

ACTA PHYSIOLOGICA SCANDINAVICA

SUPPLEMENTUM 379

TISSUE HYPEROSMOLALITY AS A
MEDIATOR OF VASODILATATION AND
TRANSCAPILLARY FLUID FLUX IN
EXERCISING SKELETAL MUSCLE

BY

JAN LUNDVALL

LUND 1972



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SKELETAL MUSCLE

AKADEMISK AVHANDLING

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SUPPLEMENTUM 379

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CHAPTER I

INTRODUCTION

There are few problems in circulatory physiology that have, in spite of intense research, resisted a final solution more than the question of the cause of exercise hyperemia. Experimental evidence strongly suggests that this vital circulatory response is causally linked to muscle metabolism and, further, that several metabolic factors contribute to the hyperemia by mutual, synergistic actions (for ref. see Barcroft 1963, Mellander and Johansson 1968, Haddy and Scott 1968).

Some years ago the hypothesis was advanced that tissue hyperosmolality produced in working skeletal muscle contributes significantly to the functional hyperemia response (Mellander *et al.* 1967). This hypothesis was supported by several later reports of *in vivo* and *in vitro* studies from our laboratories (e.g. Gray *et al.* 1968, Johansson and Jonsson 1968, Mellander and Johansson 1968, Lundvall 1969, Jonsson 1970, Mellander 1970, Mellander and Lundvall 1971).

According to this concept, exercise, through the increased metabolism, leads to formation and release of osmotically active products in muscle thereby raising the osmolality in its interstitial space, *i.e.* in the immediate environment of the vascular smooth muscle. This environmental change, in its turn, causes osmotic dehydration of the vascular smooth muscle cells; the cell shrinkage *per se* and the consequent changes of transmembrane ionic concentration gradients lead to inhibition of vascular tone and, hence, to the dilator response. The concept implies that an increased interstitial concentration of any particle would contribute to the cell shrinkage and to the vasodilatation provided it does not rapidly penetrate the cell membranes of the vascular smooth muscle.

In the present study this hypothesis will be tested along the following main lines:

The magnitude and the time-course of the osmolar changes during graded exercise will be defined and considered in relation to the concomitant vascular reactions. After this, the vascular effects of intra-arterial hypertonic infusions will be analysed when administered at rates producing similar changes of tissue osmolality in the *resting* skeletal muscle to those observed during exercise. The hyperosmolality created by hypertonic infusion to resting muscle will be denoted below as "experimental hyperosmolality". Comparison of the vascular effects in the resistance, precapillary sphincter and capacitance vessels during exercise and experimental hyperosmolality led to a quantitative evaluation of the role of tissue hyperosmolality in exercise hyperemia. These aspects are dealt with in Chapter II which also includes a critical analysis of the experimental approach to the problem. One question that proved important in this connection required

specially designed experiments and is therefore treated separately (Chapter VI). It is concerned with the problem whether during hypertonic infusion vascular smooth muscle is affected directly through the intima of the vascular wall (trans-intimal effect) or whether the effect is exerted from the interstitial space after transcapillary osmolar distribution.

In Chapter III the resistance function is analysed in greater detail insofar that the resistance changes evoked in very delimited parts of the muscle vascular tree in response to exercise and experimental hyperosmolality are defined.

In Chapter V some aspects of the interaction between the local metabolic and other vascular control systems in skeletal muscle will be considered. This type of study provided some further evidence for the hypothesis that tissue hyperosmolality is involved in exercise vasodilatation. The patterns of response to nervous and myogenic control mechanisms within the various consecutive sections of the vascular bed of skeletal muscle are known to be modified in a differentiated way by exercise (Kjellmer 1965 *a*, Lundvall *et al.* 1967). If tissue hyperosmolality is an important mediator of exercise vasodilatation it is to be expected that it will alter the nervous and myogenic response patterns in the same way as exercise. As will be shown, this criterion seems to be fulfilled.

Exercise is known to be associated with a marked net transcapillary fluid movement into the active muscles (*e.g.* Jacobsson and Kjellmer 1964). This phenomenon does not only lead to a drastic environmental change in the muscle itself in terms of a considerable fluid accumulation, but also can be of great importance for general cardiovascular dynamics since plasma volume becomes reduced. The mechanisms responsible for this capillary fluid transfer are investigated in Chapter IV. It will be shown that the fluid transfer can be ascribed mainly to an osmotic process as a result of the tissue hyperosmolality induced by work, although a filtration process as a result of increased capillary pressure contributes to the fluid flux. The processes involved in the fluid disappearance from the muscle after exercise are also investigated in this Chapter.

In Chapter VII the results from the present studies and from some others performed in the human being as well as data from *in vitro* studies on vascular smooth muscle are discussed. The data, taken together, strongly support the view that tissue hyperosmolality is an important, but not the sole, mediator of exercise vasodilatation; the experimental evidence further indicated that work induced hyperosmolality in the general circulation can establish an important osmolar control of plasma volume during exercise.

CHAPTER II

EFFECTS OF EXERCISE AND "EXPERIMENTAL HYPEROSMOLALITY" ON RESISTANCE, PRECAPILLARY SPHINCTER AND CAPACITANCE VESSELS IN SKELETAL MUSCLE

A quantitative evaluation of the role of tissue hyperosmolality in exercise vasodilatation

In this chapter an attempt will be made to analyse in detail the magnitude and time-course of the changes of tissue osmolality that occur in skeletal muscle during graded exercise and to examine to what extent these changes are correlated to the functional hyperemia response. After this, a quantitative investigation will be performed of the effects on the vascular resistance function in response to "experimental hyperosmolality" in the resting skeletal muscle produced by intra-arterial infusion of hypertonic solutions at rates causing similar changes of tissue osmolality to those in exercise. A comparative analysis of the results from these series of experiments permitted an evaluation of the role of tissue hyperosmolality in exercise hyperemia. The pattern of vascular response in the various consecutive sections of the muscle vascular bed to exercise was further reinvestigated and, partly, by a different approach than previously used. It was confirmed that work evokes a differentiated pattern of vascular response in the various sections (Kjellmer 1964 *a*); the question whether "experimental hyperosmolality" could evoke the same pattern of vascular response was finally approached.

METHODS

Material and anaesthesia: 112 cats of both sexes, weighing 2.4—5.1 kg (mean value 3.4 kg) were used in this study; (part of this material was also used in the studies reported in Chapter IV and V). Anaesthesia was induced with ether after which chloralose (50 mg/kg b.w.) and urethane (100 mg/kg b.w.) was given intravenously. Often a small dose of Nembutal (10 mg/kg b.w.) was added to depress reflex activity during surgery. Heparine (3—5 mg/kg b.w.) was given before intravascular instrumentation. Body temperature was maintained at 37.5—38.5° C throughout the experiments using a heating pad and a heating lamp.

Preparation and experimental techniques: The experiments were performed on the acutely denervated vascular bed of the isolated lower leg muscles. The preparation is essentially that described by Kjellmer (1964*a*). In brief, the skin was dissected free of the hindleg muscles down to the ankle. The lower leg musc-

les were separated from the thigh muscles and the paw was removed at the ankle. Fixation of the tendons of the lower leg muscles permitted essentially isometric muscle contractions during somato-motor fibre stimulation. The bonemarrow cavity of the femur was exposed in its distal part and plugged with cotton soaked in silicone grease to occlude any vascular connections between the femur and the calf muscles. The popliteal artery and vein formed the sole vascular connections between the studied muscle region and the main part of the body. In most experiments the muscle preparation was placed in a fixed position inside a water-filled plethysmograph (38° C), the skin of the calf, deprived of its blood supply, being replaced to cover the muscles and also used to seal the proximal entrance of the plethysmograph. With this arrangement, tissue volume could be followed continuously with the aid of a well balanced volume piston recorder connected to the plethysmograph. When no plethysmograph was used, the muscles were wrapped in gauze soaked in saline and covered by a plastic sheet to preserve a moist environment. Muscle temperature was then maintained normal using a heating-lamp.

The present study includes experiments in which blood flow was free to change ("constant pressure perfusion") and experiments in which blood flow was held constant ("constant flow perfusion"). The latter was achieved by the use of a constant perfusion pump (Harvard, Model 1210), and blood flow was then diverted from the ipsilateral femoral artery through the pump to the popliteal artery. In both types of flow perfusion, the popliteal vein was cannulated and the venous outflow from the region shunted *via* tubes to a funnel connected to the right jugular vein. In the constant pressure perfusion experiments and in some of the constant flow experiments an optical drop-recorder unit was inserted in the venous shunt for continuous recording of the blood flow. In most of the constant flow experiments the drop-recorder was instead inserted in the shunt on the arterial side. Mean arterial inflow pressure was monitored from a cannula in the contralateral femoral artery during constant pressure perfusion. During constant flow perfusion, it was monitored from a T-tube in the arterial shunt distal to the pump. By adjusting the height of the orifice of the outflow tubing from the muscle region, venous outflow pressure could be controlled. In the plethysmographic experiments venous outflow pressure was adjusted so as to produce an isovolumetric state in the resting control period (venous outflow pressure 5—8 mm Hg).

Blood flow, arterial pressure and tissue volume were recorded on kymograph paper or on a Grass Polygraph.

In experiments in which intra-arterial infusions (or injections) were given, special attention was paid to the problem of thorough mixing with blood. In the constant flow experiments this could be achieved by giving the infusions in the tubing proximal to the pump. During constant pressure perfusion thorough mixing was accomplished by inserting a specially designed mixing chamber (Fig. 1) in an arterial shunt circuit connecting the ipsilateral femoral artery to the pop-



Fig. 1. Schematic drawing of "mixing chamber" used to produce turbulence and thereby thorough mixing of blood and intra-arterially infused solutions. The chamber was inserted in a short shunt circuit diverting flow from the femoral to the popliteal artery. Scale 1:1.

liteal artery. The chamber was made of inert plastic material and of silicone tubing. Dye infusion test experiments in which this chamber was perfused with water at similar flow rates to those encountered in the *in vivo* experiments showed that by producing turbulence the chamber caused thorough mixing. Mixing was, however, highly inadequate without using the chamber. Although the viscosity of blood differs from that of water it appears likely that the results of these test experiments are valid for the present *in vivo* experiments. The results below strongly suggest that homogeneous distribution of an infused solution in the muscle vascular bed was only obtained by the use of the mixing chamber and, further, that the mixing chamber did not cause aggregation of blood cells or liberation of vasodilator agents in the blood (see Comments). The pressure drop across the mixing chamber was found to be insignificant even at flow rates exceeding those encountered in the present study. This was checked in the *in vivo* situation by pressure recordings during blood perfusion at varying rates produced by a pump. The resistance of the whole arterial shunt circuit was similarly found to be quite insignificant.

Muscle exercise (single twitches) was produced by electrical stimulation of the distal end of the severed sciatic nerve using a Grass stimulator, model S4 K. Supramaximal stimuli (5V, 0.5 msec) at a frequency of 0.25—4 imp/sec were delivered. That the sympathetic fibres are not excited by such stimuli applied for somato-motor fibre activation has previously been shown (*e.g.* Kjellmer 1964a) and was confirmed in the following way. The muscles were first shown to contract upon sciatic nerve stimulation and the concomitant exercise hyperemia was observed. When the blood flow had returned to the control level after work, gallamine iodide (Flaxedil) was given intra-arterially to block the neuromuscular junction, and the nerve stimulated again as before. No contractions and no blood flow change was now observed showing that the autonomic fibres were not excited. However, a further increase of the duration and/or voltage of the stimuli produced a marked increase of vascular resistance, showing that the adrenergic fibres could indeed be activated by stronger stimuli.

In six animals the isometrically developed muscle force during exercise was measured using a force transducer (Grass, Model Ft 10) attached to the Achilles tendon. In these experiments the leg was fixed rigidly by pins inserted into the femur and tibia.

Isotonic and hypertonic (900—1700 mOsm/kg H₂O) solutions were infused into the arterial shunt at rates between 0.1 and 1.9 ml/min using a constant infusion apparatus. Solutions of glucose or sodium lactate were generally used, but sucrose, xylose, mannitol, urea and NaCl were also tested. The solutes were dissolved in distilled water.

For measurement of venous and arterial plasma osmolality blood samples (<1.5 ml) were taken from a T-tube in the venous outflow tubing close to the popliteal vein and from a catheter in the brachial artery (exercise experiments), or from a T-tube in the arterial shunt distal to the mixing chamber (hypertonic infusion experiments) after discarding the "dead space" fluid volumes. Such samples were withdrawn before, during and after exercise and hypertonic infusion. After centrifuging, plasma osmolality was determined by thermistor cryoscopy (Osmometer 31 LAS, Advanced Instruments, Inc.). Each sample was measured twice. When different readings were occasionally obtained, the mean value was used. Readings in repetitive measurements on osmolar standards deviated at most by 1.5 mOsm/kg H₂O from the true value. After osmolality had been determined, the sampled blood was returned to the animal. When several blood samples were taken in rapid succession any significant change in blood volume was avoided by the following procedure. A suitable amount of an isotonic dextran-Tyrode's solution was given i.v. at least 10 min before the experiment, a time period sufficient for thorough mixing in the general circulation. Concomitantly, a corresponding amount of blood was withheld extracorporeally in the funnel connected to the external jugular vein. By graded return of the extracorporeal blood during subsequent blood sampling the intravascular blood volume was kept approximately constant.

With the present experimental approach it was possible to follow the influence of exercise and of hypertonic infusion on the resistance vessels, precapillary "sphincters", and capacitance vessels as well as on net transcapillary fluid movement (for detailed discussion see Mellander 1960, Mellander and Johansson 1968). The resistance function was given by the pressure flow relation. Changes in precapillary sphincter activity, determining the number of patent capillaries and, hence, the functional capillary surface area available for transcapillary exchange, were followed by determination of the capillary filtration coefficient (CFC). It was assumed that no change in capillary permeability occurred in response to exercise or hypertonic infusion (see Comments). CFC was determined by recording the rate of net transcapillary fluid filtration caused by raising venous outflow pressure by a known amount (see Mellander 1960, Cobbold *et al.* 1963). For the CFC calculations, it was assumed that 80 % of the venous pressure rise was transmitted to the capillary level during constant pressure perfusion (*cf.* Cobbold *et al.* 1963) and 100 % during constant flow perfusion (*cf.* Kjellmer 1964 a).

The level of vascular tone present in the capacitance (mainly venous) vessels in the control state and during exercise and hypertonic infusion was determined

by observing the rapid tissue volume increase (reflecting changes of regional blood volume; see Mellander 1960, Åblad and Mellander 1963, Mellander and Johansson 1968) caused by a short i.a. infusion of a supramaximal dose of acetylcholine ($> 100 \mu\text{g/kg}$ muscle). This substance is known to cause a virtually maximal dilatation of the muscle capacitance vessels (Åblad and Mellander 1963, Mellander 1966). These experiments were performed under constant flow perfusion to avoid any significant passive capacitance effect due to changed distending pressure in the venous section. In some experiments the capacitance effects were recorded concomitantly by external monitoring regional changes in the radioactivity of the red cells labelled with ^{51}Cr (for details see Åblad and Mellander 1963, Kjellmer 1965a).

Acetylcholine was also used as a reference substance to induce a short maximal dilatation of the resistance vessels and was given as a slow i.a. injection in a dose exceeding $100 \mu\text{g/kg}$ muscle (*cf.* Kjellmer and Odelram 1965). In order to produce a stable, long-lasting maximal resistance vessel dilatation, papaverine was infused intra-arterially ($> 0.6 \text{ mg/min}$).

For analysis of changes of organic and inorganic constituents of plasma during exercise, arterial and regional venous blood samples were withdrawn as described above. 5 % CO_2 was added to the gas phase of the samples to prevent chloride shift between plasma and erythrocytes. The blood samples were immediately centrifuged and the plasma to be used for determination of organic constituents frozen in liquid nitrogen. The plasma concentrations of lactate, pyruvate and glucose were measured with specific enzymatic (fluorometric) techniques after extraction of the plasma samples at -25°C (see Lowry *et al.* 1964, Goldberg *et al.* 1966). The Na^+ , K^+ and Ca^{++} concentrations were measured using conventional plasma photometry (Eppendorff). The plasma chloride concentration was measured using the Cotlove chloride analyser (American Instruments Co., USA). pO_2 , pCO_2 and pH were determined on blood samples withdrawn anaerobically using microelectrodes (Eschweiler and Co., Kiel, and Radiometer, Copenhagen). The plasma bicarbonate concentration was calculated from pCO_2 and pH using the Siggaard-Andersen nomogram (1963).

After each plethysmographic experiment (majority of the experiments) the lower leg muscles were dissected free from the bones for determination of muscle tissue weight. The mean weight of the muscles, after correction for any volume changes during the experiments, was $16.9 (\pm 1.4 \text{ SD}) \text{ g/kg b.w.}$ In the other experiments this calculated mean value was used to estimate the mass of the studied muscle group from the body weight.

RESULTS

A. Resistance vessels

Attempts to reveal in quantitative terms the causal role of any factor in the functional hyperemia response in skeletal muscle must primarily be directed to an analysis of its effects on the resistance function. Experimental approaches aimed at disclosing the mechanisms responsible for exercise hyperemia usually imply studies under conditions that more or less deviate from the normal situation. Such circumstances, besides the normal biological variation, can lead to considerably varying results in different experiments. It follows that reliable conclusions may not be reached unless the number of observations is sufficiently large, a demand believed to be satisfied in the present study.

The results concerning the resistance vessels will be presented in three sections. In the first two (A1, A2), the general characteristics of the resistance responses and the concomitant osmolar changes during exercise and experimental hyperosmolality will be outlined. An attempt is also made to critically evaluate possible experimental errors. In the last section (A3) the collected data on the resistance changes versus the osmolar changes during exercise and experimental hyperosmolality are presented on a comparative basis to permit an evaluation of the role of hyperosmolality for the exercise hyperemia response.

The plasma osmolality of arterial blood and of the regional venous blood was, in the control period at rest, usually of about the same order of magnitude, the venous value, however, sometimes being slightly higher (1—2 mOsm/kg H₂O). Venous plasma osmolality, reflecting interstitial osmolality, varied to some extent between animals and averaged 318 ± 5 SD mOsm/kg H₂O at the time the surgical procedures were finished.

In the individual animal, resting control venous (interstitial) osmolality at times exhibited a slow, progressive increase from the initial value in experiments involving several periods of exercise or hypertonic infusion. According to the hypothesis dealt with, an increased interstitial osmolality should elicit vasodilatation through osmotic shrinkage of the vascular smooth muscle. Such shrinkage can be expected to be most clear-cut in response to acute changes of interstitial osmolality, but may tend to disappear with time due to solute penetration into the smooth muscle (*cf.* Comments). Therefore the effects of more long-termed osmolar changes occurring over a time period of several hours might be negligible. The variation in steady state control osmolality was not found to influence control resistance which is consistent with this view.

It appears predictable that a change of vascular smooth muscle volume and a thereby ensuing vasodilatation in response to a given acute increase of interstitial osmolality should be related to the prevailing osmolality in the control period before the experimental intervention. However, the variation in control osmolality in the present study was small and such a relation was not revealed.

led. The vascular effects described below will therefore only be related to the evoked changes of osmolality from the preceding control level; absolute figures for total osmolality will thus not be used.

1. Changes of resistance and of plasma osmolality during exercise

The muscles of the lower leg, except for the small soleus muscle, mainly consist of "white, phasic" fibres. Although the physiological motor fibre discharge to a muscle like the gastrocnemius can amount intermittently to 50 to 60 impulses per sec, it is well known that a maximal resistance vessel dilatation is elicited at electrical nerve stimulation rates of 4 to 5 impulses per sec (e.g. Kjellmer 1964 a, Folkow and Halicka 1968), an observation confirmed in the present investigation. At higher stimulation rates blood flow is impeded owing to mechanical interference of the muscle contraction (e.g. Folkow and Halicka 1968). In work experiments designed to reveal the role of a metabolic factor in exercise hyperemia such low stimulation rates should therefore be used so as to produce single twitches. The experiments were performed both under constant pressure perfusion when, as in the intact organism, blood flow is free to change as well as under constant blood flow perfusion. In most of the experiments the more physiological condition of constant pressure perfusion was used and these experiments will be described first. For reasons discussed below (Comments section) a dilator response during constant pressure perfusion will be expressed in terms of the evoked change in vascular conductance (flow/pressure) whereas, during constant flow perfusion, it will be expressed as the evoked change of the flow resistance (pressure/flow).

Fig. 2 illustrates an experiment in which the changes in blood flow and in plasma osmolality evoked by exercise at 4 imp/sec were followed. In the control period at rest, blood flow was about 13 ml/min $\times$ 100 g tissue and arterial and venous osmolality were equal (316 mOsm). It can be seen that blood flow started to increase very shortly after the commencement of exercise and, within some 2 min, it reached about a maximal level of 45 ml/min $\times$ 100 g which was then maintained during the period of work. Similarly, venous osmolality rose quickly after beginning of exercise, as shown by the blood sample taken after 20–25 sec, and the hyperosmolality reached about a maximal value of 22 mOsm above the control level after some 2.5 min and was then maintained at this level during the period of work. Since arterial osmolality was not altered significantly it is evident that the osmolar change emanated from the exercising muscles, apparently caused by production and release of osmotically active particles by the contracting striated muscle fibres. The change of venous osmolality thus reflects indirectly, and with some delay, the hyperosmolality created primarily in the extravascular compartments. It follows that hyperosmolality in the interstitial space, the immediate environment of the vascular smooth muscle, is established in a very early phase of work. At the cessation of the exercise, both blood flow and venous osmolality gradually returned towards the control levels. This expe-

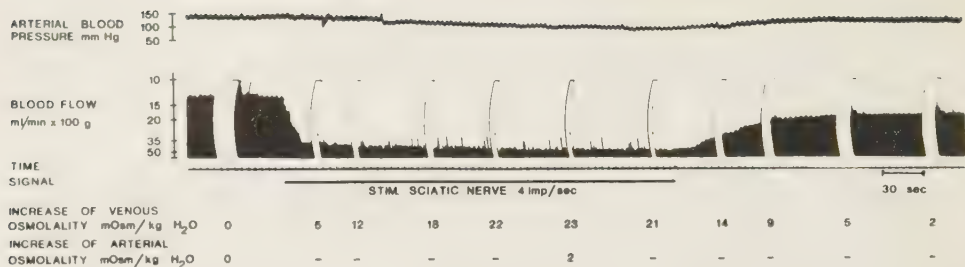


Fig. 2. Osmolar changes in venous effluent from cat skeletal muscle during and after short-term (~ 5 min) "heavy" exercise (somato-motor fibre stimulation 4 imp/sec). Blood flow increased rapidly to reach a maximal level of 45 ml/min $\times$ 100 g after about 2 min. Venous osmolality, reflecting interstitial osmolality, started to rise soon after beginning of exercise and reached a maximal maintained increase (~ 22 mOsm/kg H₂O) within 2.5 min. Arterial osmolality virtually unchanged indicating that the venous hyperosmolality emanated from the exercising muscle. Upon cessation of exercise, blood flow and venous osmolality returned gradually, and in parallel, towards the control levels.

riment strongly suggests that there is a close co-ordination between the osmolar and the vascular changes during and after exercise.

The changes with time of the blood flow and venous osmolality were analysed in 23 experiments similar to the one described above, but performed at varying motor nerve stimulation rates (0.25 to 4 imp/sec). Within this frequency range, graded exercise was produced which, with regard to both resistance and osmolar effects, ranged from barely discernible to maximal. For the sake of simplicity, exercise evoked by rates less than 1/sec will be classified below as "light", by 1 to 2/sec as "moderate", and by 3 to 4/sec as "heavy". The exercise hyperemia usually reached a steady state within 60 to 120 sec, the lower figure being more representative for heavy work and the higher more representative for light work. An increased osmolality in the venous effluent was usually observed within less than 30 sec after the commencement of exercise and the hyperosmolality attained a maximal level within 2 to 4 min in the great majority of the experiments. Like the hyperemia response, the venous osmolar change reached a maximum earlier at heavy work than at light work. After exercise, flow and venous osmolality returned to the control levels and largely in parallel. The recovery period varied in different experiments but usually did not exceed 20 min. It should be stressed that the osmolar changes in the interstitium must be more closely co-ordinated in time with the described vascular changes than the above data indicate since the osmolar events in the venous effluent inevitably lag somewhat behind those in the extravascular space, *e.g.* because of the transit time from capillary to the place of venous sampling. Especially in the initial phase of exercise when the hyperemia is not yet developed such time lag must be of significance.

From the experiments described, it is clear that, regardless of the work intensity, both the changes in vascular resistance and in venous osmolality can be

considered fully developed after 3—4 min of exercise ("steady state phase of exercise"). This implies that the question whether there is a quantitative correlation between the hyperemia response and the venous hyperosmolality can be approached by comparing these phenomena during relatively short (<5 min) exercise experiments. Such an analysis was made in 104 experiments performed at varying work intensities. Two different experimental procedures were applied. In one type, graded exercise was produced by step-wise increase of the rate of motor nerve stimulation so as to elicit 2 to 3, occasionally 4, consecutive work periods, each of 4 to 5 min duration. In the other, the work periods were separated in time so as to permit full recovery of the vascular and osmolar changes in between periods of graded stimulation. No major difference could be found between the results from these two types of experiment.

There was in general, and in particular in the individual experiment, a clear relationship between the rate of motor nerve stimulation (work "intensity") and the extent to which blood flow and venous osmolality rose above the control values in the steady state phase of exercise. During heavy work (4 imp/sec), muscle blood flow was on the average $42 \text{ ml/min} \times 100 \text{ g tissue}$ at a perfusion gradient of about 100 mm Hg, but not infrequently it reached some $60 \text{ ml/min} \times 100 \text{ g}$. With few exceptions, the observed flow values at 4 imp/sec represented a maximal, or close to maximal resistance vessel dilatation, as tested by close arterial acetylcholine injection (see Methods). At this stimulation rate, the increase of venous osmolality averaged 22 mOsm, but occasionally amounted to 40 mOsm.

The original record in Fig. 3 may serve to illustrate the mentioned relation between the hyperemia and venous hyperosmolality at graded stimulation rates (signal). It can be seen that at a perfusion pressure of about 100 mm Hg (venous outflow pressure 6 mm Hg) blood flow increased step-wise with increasing work intensity from a resting control value of 7.3 to a maximal value at 4 imp/sec of $45.3 \text{ ml/min} \times 100 \text{ g tissue}$. The observed increase of venous osmolality and the calculated changes of vascular conductance in the different exercise periods are shown at bottom of the figure. It can be seen that during exercise these two parameters were closely correlated. In the postexercise period, vascular conductance and venous osmolality gradually waned, and roughly in parallel, down to the control levels, which were reached in about 20 min.

Fig. 3 also illustrates the increase of skeletal muscle volume caused by net transcapillary movement of fluid from blood to the active muscles during work. This process will be described in detail below (Chapter IV). One aspect of the transcapillary fluid movement will however be considered here, as it is of relevance for the blood flow determinations. When blood flow, as in the present experiments, is recorded as the venous outflow from the muscles, the transcapillary fluid loss implies that blood flow in the precapillary vessels is somewhat underestimated. The rate of fluid movement was related to the stimulation frequency, as can be seen in Fig. 3. The maximal rate of fluid flux thus occurred at

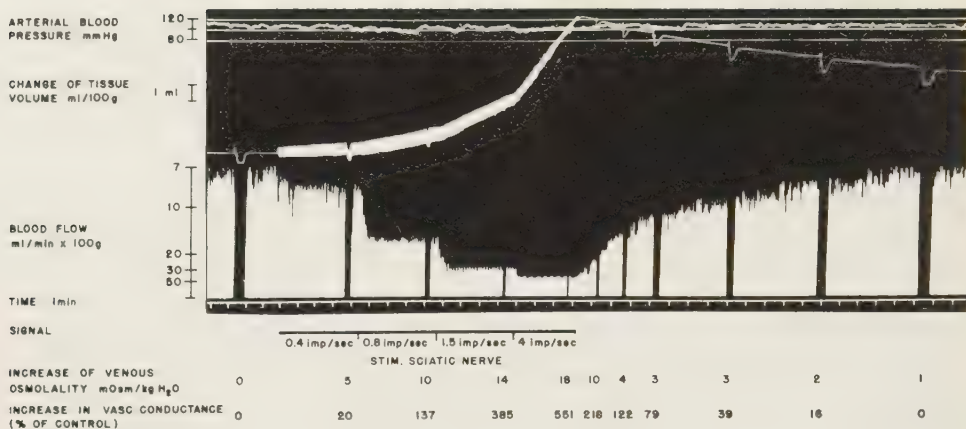


Fig. 3. Changes of vascular conductance and venous osmolality during and after graded skeletal muscle exercise. Note close correlation between the vascular and osmolar events. Tissue volume curve shows increasing net transcapillary fluid movement into muscle with increasing motor nerve stimulation rate.

4 imp/sec and amounted here to 1.7 ml/min x 100 g tissue, corresponding to 3.8 % of the concomitant venous blood flow (45.3 ml/min x 100 g). At the lower work intensities, this percentage was smaller. In none of the exercise periods in which this fluid loss was recorded (as was the case in the majority of the experiments) did it exceed 5 % of the concomitant venous blood flow. It follows that during work the total resistance function in the muscle vascular bed can be calculated with quite reasonable accuracy from observations of venous outflow.

Collected data from all exercise experiments ($n=104$) performed at constant pressure perfusion showed a statistically highly significant correlation between the following parameters: 1. stimulation frequency (0.25—4 imp/sec) and observed per cent increase in vascular conductance ($r=0.70$, $p<0.001$), 2. stimulation frequency and degree of venous hyperosmolality ($r=0.78$, $p<0.001$), and 3. per cent increase in vascular conductance and degree of venous hyperosmolality ($r=0.51$, $p<0.001$).

As will be discussed in section A3, the quantitative evaluation of the role of a metabolic factor in exercise hyperemia might be erroneous if the results from experiments are included in the material in which the preparations in the control period exhibited a resistance vessel tone that deviated far from normal. In the processing of data such errors can be avoided by excluding, on a statistical basis, experiments with extreme control resistance values. Fig. 4 is a diagram based on the 83 exercise experiments in which this type of analysis indicated that the resting tone of the resistance vessels could be considered normal (see A3). The increase of vascular conductance, expressed in per cent of the control value, is plotted against the concomitant venous hyperosmolality. The

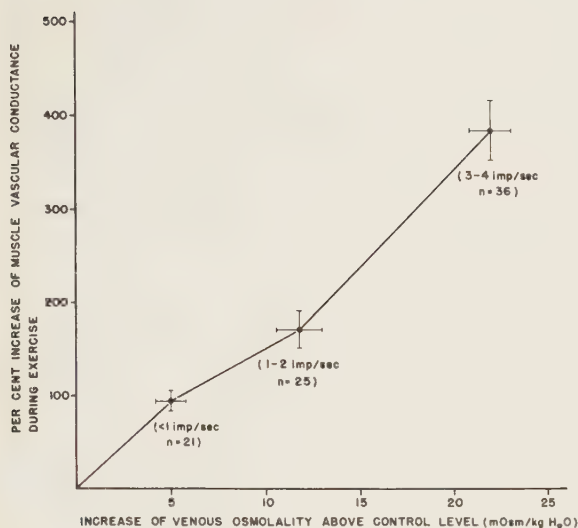


Fig. 4. Diagram showing increase of muscle vascular conductance versus the concomitant increase of venous osmolality during graded exercise ($n=83$). Data, classed with regard to rate of somato-motor fibre stimulation ("work intensity"), refer to observations 2—4 min after commencement of exercise when vascular and osmolar changes were fully developed. Highly significant correlations existed between stimulation frequency and increase of vascular conductance, between stimulation frequency and degree of venous hyperosmolality, and between conductance increase and degree of venous hyperosmolality.

data are classed with regard to the rate of somato-motor fibre activation and the mean values $\pm$ SEM are given. It can be seen that the increase of vascular conductance and the degree of venous hyperosmolality during exercise were related to each other also in this material and that both these parameters, in turn, showed a relation to the stimulation frequency.

A possible dilator action of the work induced hyperosmolality on the vascular smooth muscle should be exerted from the interstitial space. As a crude approach to the problem whether the hyperosmolality in this tissue compartment was correctly reflected in the venous effluent the following type of experiment was performed. Heavy muscle exercise was produced (4 imp/sec) and a venous blood sample was taken 3—4 min after the commencement of work, *i.e.* in a phase when venous hyperosmolality should have reached its maximal level (see above). Blood flow was then immediately reduced to about 1/10 of that during the hyperemia response with the aid of a screw-clamp placed around the abdominal aorta and repetitive venous blood samples were withdrawn. On average venous hyperosmolality in the phase of reduced flow, when the transcapillary osmolar equilibration should be improved as a result of the decreased capillary flow velocity, exceeded that in the unrestricted hyperemia phase by 12 per cent (mean of 9 experiments), suggesting that a complete transcapillary osmolar equilibrium may not be established during exercise.

In the human being the post-exercise hyperemia is known to vanish in an approximately exponential manner (Dornhorst and Whelan 1953, Hyman and Wong 1968). In 39 of the present exercise experiments of varying intensity and

in which arterial blood pressure remained constant, the post-exercise excess flow (post-exercise flow minus resting control flow) was plotted semi-logarithmically against time. A strictly exponential blood flow decay, with a mean half-time ($T_{1/2}$) for flow restoration to the control level of $118 (\pm 24 \text{ SEM})$ sec, was found in 27 of these experiments. $T_{1/2}$ was not correlated to the degree of exercise hyperemia. The value for $T_{1/2}$ after exercise will be compared with the $T_{1/2}$ value after hypertonic infusion (A 2).

The results hitherto described refer to observations made during exercise of relatively short duration (<5 min). Changes of resistance and of venous osmolality were also studied during longer work periods, usually of 15 min duration, which below is denoted "prolonged" exercise. During prolonged work, the hyperemia at all stimulation rates usually remained close to its maximal level reached within a few minutes after the commencement of work. At light and moderate exercise, the venous hyperosmolality also generally persisted at its maximal value throughout the prolonged work periods. At heavy work, however, venous osmolality showed in virtually all experiments a progressive decline with time from its peak value, but always stayed well above the control level at rest. In 13 exercise experiments at 4 imp/sec, the venous hyperosmolality values after 2 to 4 min and after 15 min of work thus were different ($p < 0.001$), the mean increases being $22 \pm 2 \text{ SEM}$ and $12 \pm 3 \text{ SEM mOsm}$, respectively.

The declining venous hyperosmolality during heavy exercise seemed to occur in parallel with a gradually decreasing muscle contraction force. The latter phenomenon was attested in a crude way by mere inspection of the contracting muscles and, further, by analysis of the amplitude of the twitch recordings as reflected in the muscle volume tracing. To verify these indirect observations, calf muscle isometric tension development during exercise was measured in 6 animals by a force transducer attached to the Achilles tendon. Table I shows the mean changes in vascular conductance, venous osmolality, and muscle tension observed in various time periods of prolonged light (0.5 imp/sec) and heavy (4 imp/sec) work. It can be seen that during light exercise the vascular conductance, the osmolality, and the muscle tension remained about constant throughout the exercise period once the responses were developed. During heavy work on the other hand, venous hyperosmolality and muscle tension after attaining their peak values both showed a gradual decline to significantly lower levels after 15 min whereas no significant reduction of the hyperemia response was revealed. Note, however, that venous osmolality after 15 min was still clearly above the control level. The drastic decline with time of muscle tension may indicate that exercise produced by nerve stimulation at 4 imp/sec for a *prolonged* period of time represents a "supra-maximal" work which cannot be performed in the intact organism for more than a few minutes. The gradual decrease of venous hyperosmolality during prolonged work at 4 imp/sec, in turn, may therefore possibly be sort of an "experimental artefact".

When, in the same animal, a prolonged exercise was repeated after a resting

Time after commencement of exercise:		20 sec	50 sec	3 min	7 min	15 min
Increase of vasc. conduct- tance (% of control)	0.5 imp/sec	23 ± 5	55 ± 4	98 ± 14	123 ± 23	118 ± 30
	4 imp/sec	191 ± 56	303 ± 76	394 ± 59	407 ± 75	416 ± 83
Venous hyperos- molality (mOsm/ /kg H ₂ O)	0.5 imp/sec	1.0 ± 0.3	1.8 ± 0.5	4.5 ± 0.5	5.4 ± 0.9	6.4 ± 1.4
	4 imp/sec	2.7 ± 0.4	11.7 ± 0.8	22.0 ± 0.9	17.2 ± 1.3	10.2 ± 0.3
Muscle force (g)	0.5 imp/sec	771 ± 195	873 ± 205	911 ± 199	911 ± 196	893 ± 126
	4 imp/sec	863 ± 118	858 ± 122	469 ± 69	285 ± 77	168 ± 58

TABLE I. Mean data $\pm$ SEM for vascular conductance increase, venous hyperosmolality, and muscle force during prolonged "light" (somato-motor fibre stimulation 0.5 imp/sec) and "heavy" (4 imp/sec) exercise. During light exercise these parameters remained about constant throughout the period of work once the effects were developed. During heavy exercise venous hyperosmolality and muscle force after attaining peak values showed a gradual decline whereas the conductance increase was maintained.

period more than sufficient for recovery of blood flow and of venous osmolality to the control levels, the elicited changes of vascular conductance, venous osmolality, and muscle tension were about equally large in the second period of light exercise, whereas the latter two effects were somewhat reduced, but never disappeared in the second period of heavy exercise. The last-mentioned tendency was not revealed in repetitive short-term (<5 min) exercise, nor did the degree of venous hyperosmolality seem to be affected when several sequences of graded brief work evoked in a step-wise fashion were produced.

Arterial osmolality remained largely unchanged at its control level during light exercise even if prolonged. During heavy exercise, however, arterial plasma osmolality increased gradually, by some 1—3 mOsm after 3—4 min, and by 3—10 mOsm after 15 min. This increase may mainly be explained by delivery of osmotic products from the contracting lower leg muscles to the general circulation, although, for instance, an increased glucose concentration, not emanating from the active muscles, can contribute (see below). From observations on the

	Rest	4 min of exercise	15 min of exercise
Osmolality mOsm/kg H ₂ O	321.3 ± 2.3	337.5 ± 7.9 +16.2	332.3 ± 4.8 +11.0
Na ⁺ mEq/L	151.3 ± 0.7	158.7 ± 1.2 +7.4	153.0 ± 3.5 +1.7
K ⁺ mEq/L	4.3 ± 0.3	6.3 ± 0.5 +2.0	5.3 ± 0.7 +1.0
Lactate mM/L	1.6 ± 0.6	5.1 ± 1.5 +3.5	6.5 ± 1.9 +4.9
Glucose mM/L	6.8 ± 0.07	8.6 ± 0.8 +1.8	9.8 ± 3.0 +3.0
HCO ₃ ⁻ mEq/L	24.6 ± 1.9	23.8 ± 1.6 -0.8	21.9 ± 1.8 -2.7
pO ₂ mm Hg	41 ± 7	19 ± 5	16 ± 3
pCO ₂ mm Hg	56 ± 10	69 ± 11	76 ± 15
pH	7.276 ± 0.065	7.179 ± 0.046	7.103 ± 0.061

TABLE II. Data from analyses on venous effluent from muscle during "heavy" (4 imp/sec) exercise (3 exp.). For description see text.

veno-arterial osmolar difference and the blood (plasma) flow it was apparent that the osmotic delivery was maximal in the time period 2—4 min after onset of exercise, and then amounting to some 0.4—1.0 mOsm/min, after which it gradually declined. These observations seem roughly compatible with the figures for arterial hyperosmolality, taking into consideration the distribution of the osmolar products primarily in the intravascular compartment and later in extra-vascular fluid of inactive tissues.

In order to get some information about the substances responsible for the interstitial hyperosmolality in exercising muscle, the venous plasma concentrations of a number of organic and inorganic substances and venous plasma osmolality were determined in the control period at rest and after about 4 and 15 min of heavy work (4 imp/sec) in three animals. The pO_2 , pCO_2 , and pH were also measured. Corresponding analyses were performed on arterial blood. The following substances were analysed: lactate, pyruvate, glucose, sodium, potassium, calcium, chloride and bicarbonate. Of these substances only those listed in Table II (mean values $\pm$ SEM), showed changes of such magnitudes as to be of significance for the concomitant osmolality changes during work. The changes of pO_2 , pCO_2 and pH are also shown and these data indicate that the work, which on average increased flow from a control value of 7 to a maintained level of $36 \text{ ml/min} \times 100 \text{ g}$, was quite "heavy". Four minutes after the commencement of work venous plasma osmolality was raised by $16 \text{ mOsm/kg H}_2\text{O}$. Concomitantly the plasma concentration of sodium was increased by 7.4 mEq/lit , potassium by 2.0 mEq/lit , lactate by 3.5 mM/lit , and glucose by 1.8 mM/lit . The bicarbonate concentration, on the other hand, was reduced by 0.8 mEq/lit . The sum of these concentration changes would, in approximate terms, correspond to an osmolality increase of about 14 mOsm , *i.e.* to almost 90 % of the observed osmolar change; (since the osmotic coefficients of the substances are below unity this figure, however, represents an overestimation). Sodium and lactate seem mainly responsible for the venous (interstitial) osmolality increase. Glucose was the only of these substances that was significantly increased in the arterial blood and the changes of the others emanated from the exercising muscle. The increase of sodium tentatively can be ascribed to a passive effect of fluid (water) flux from the interstitial space into the contracting skeletal muscle cells (*cf.* Chapter IV).

After 15 min of work the osmolality increase averaged 11 mOsm . Simultaneously, the concentration of sodium was increased by 1.7 mEq/lit , potassium by 1.0 mEq/lit , lactate by 4.9 mM/lit , and glucose by 3.0 mM/lit . The bicarbonate concentration was reduced by 2.7 mEq/lit . The sum of these concentration changes corresponds to an osmolar increase of some 8 mOsm . These figures suggest that in later phases of "prolonged heavy" work, lactate was of special importance for the evoked osmolar change.

The experiments ($n = 59$) in which blood flow was held constant during exercise supported in essential parts the results arrived at in the "constant perfusion" studies, although some quantitative differences were present. Graded exercise thus led to graded dilatation of the resistance vessels (drop in perfusion pressure) and to graded venous hyperosmolality. There was, however, relatively little difference in the resistance response at stimulation rates between 2 and 4 imp/sec, whereas the venous hyperosmolality was always greater at the latter rate of stimulation. This difference may be explained by the fact that maximal dilator responses in the resistance vessels are difficult to reveal by the constant flow method (see Comments). At a given stimulation rate the osmolar change

in venous blood was developed somewhat earlier and tended to reach higher levels than in the experiments with constant pressure. Thus, at 4 imp/sec, venous osmolality could rise to values above 40 mOsm, the maximal value being 47 mOsm/kg H₂O. During prolonged work the hyperosmolality was found to be better maintained under constant flow perfusion than under constant pressure perfusion, a difference which partly might be related to a greater wash-out of osmolar products from the muscle during the latter type of perfusion. After 10 min of heavy work under constant flow the venous hyperosmolality thus roughly equalled that observed 2—3 min after the commencement of exercise (n=6).

2. *Changes of resistance evoked by experimental hyperosmolality in resting skeletal muscle.*

The results presented in the previous section strongly indicated that the degree of hyperosmolality created in the extravascular space of muscle during work is correlated to the magnitude of the exercise hyperemia response; hyperosmolality thus fulfills a prime criterion of a metabolic factor involved in this reaction. In order to analyse in quantitative terms the effects of hyperosmolality *per se* on the resistance function, hypertonic solutions were infused close arterially to the resting skeletal muscle so as to produce an "experimental hyperosmolality" of the tissue in the range encountered in exercise. The osmolar changes during exercise were found to develop rapidly and were fully established within a few minutes after the commencement of work. The analysis of the resistance effects of experimental hyperosmolality should be performed in such a way that these osmolar changes are resembled with regard to magnitude and time course in order to be relevant for the present problem, the role of hyperosmolality in exercise hyperemia. The main emphasis will therefore be placed on results obtained in short term hypertonic infusion experiments in which relatively rapid changes of tissue osmolality were produced.

Tissue hyperosmolality during hypertonic infusion evidently is accomplished *via* transcapillary osmotic exchange, but, as for the exercise experiments in the previous section, the degree of the induced osmolar change in the extravascular space cannot be determined directly, but merely as it is reflected in the venous effluent. The hyperosmolality of venous plasma probably exceeds that created in the tissue during short term hypertonic infusion (see below). It seemed necessary, therefore, to study the vascular effects over a venous hyperosmolality range exceeding that observed in exercise. Hypertonic solutions of glucose or sodium lactate were mainly used, but sucrose, sodium chloride, urea, mannitol and xylose solutions were also tested, *i.e.* both metabolizable and non-metabolizable substances. The use of sodium lactate was prompted by the finding that venous hyperosmolality of exercise could mainly be attributed to increased concentrations of the sodium and lactate ions (Table II). The effects to be described can be considered as caused by changes of osmolality *per se* and not by more specific actions on the vasculature of the solutes used, since routine control infusions of isotonic

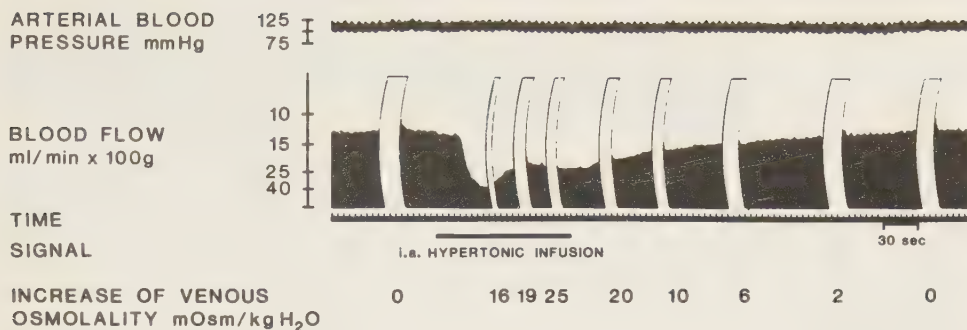


Fig. 5. Blood flow changes produced in resting skeletal muscle by intra-arterial infusion of hypertonic sodium lactate solution — (creating “experimental hyperosmolality”). Flow increased rapidly to a peak value and then decreased during continued infusion to attain an approximate steady level of hyperemia. The observed increase of venous osmolality above control level during the “peak flow response” and the “steady state flow response” is shown. After cessation of infusion flow and venous osmolality returned to control levels in parallel.

solutions obviously creating concentrations in the tissue of the respective solutes far above normal, gave no significant vascular effects. These isotonic infusions, performed at the same rates as the hypertonic ones, also served as control experiments to test the rheological effects of the fluid infusion *per se*. These became significant only at the higher infusions rates, and it should be stressed that they have been corrected for in all diagrams below depicting the resistance effects of experimental hyperosmolality.

The resistance effects of hypertonic infusion observed both during constant pressure perfusion and constant flow perfusion will be described in relation to the induced venous hyperosmolality. As in the exercise section above, the general characteristics of the response as well as some quantitative aspects will be considered first. After this, a critical analysis of possible mechanisms behind these resistance effects will be presented which indicates that they can almost entirely be ascribed to a direct effect on the vasculature in terms of an active inhibitory smooth muscle response.

The general characteristics of the vascular response to experimental hyperosmolality were similar for all substances used, except for urea. The effects of urea will therefore be considered separately later on.

Fig. 5 illustrates the most commonly observed pattern of blood flow response during experimental hyperosmolality, in this case caused by sodium lactate infusion. The pressure head was approximately constant (about 110 mm Hg) throughout the experiment. Blood flow was about 12 ml/min x 100 g tissue in the control period and it increased shortly after the commencement of the hypertonic infusion to reach a maximal value of about 36 ml/min x 100 g within some 20 sec, at which time venous osmolality had risen by 16 mOsm/kg H₂O. Below,

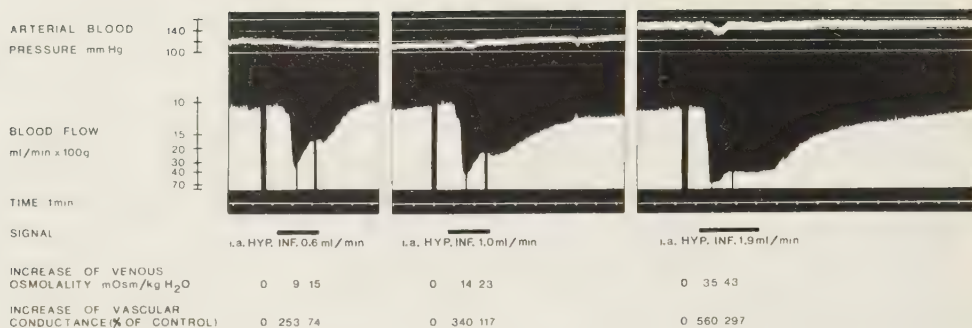


Fig. 6. Vascular conductance changes in response to graded "experimental hyperosmolality" in resting skeletal muscle.

this flow effect will be denoted the "peak response". Blood flow then showed a clear tendency to decrease and it attained an approximate steady state hyperemia level (23 ml/min $\times$ 100 g) about 60 sec after the commencement of the flow response, and this hyperemia was maintained during the rest of the infusion period (below denoted "steady state response"). Venous osmolality increased by 19 mOsm in the early phase of the steady state response and then showed some further increase. Upon cessation of the hypertonic infusion blood flow and venous osmolality waned gradually, and in parallel, towards the control values.

In the vast majority of 160 similar experiments in which the hypertonic infusion rate was varied so as to produce graded levels of venous hyperosmolality, a similar flow pattern was observed. In this material, (urea infusions not included, see below), the peak flow was reached within 15 to 50 sec after the commencement of the response and the steady state flow after another 30 to 90 sec. The flow changes tended to appear earlier at high than at low levels of venous hyperosmolality. In some 10 per cent of these short term infusions experiments, especially when either quite small or quite large changes of venous osmolality were evoked, the flow pattern deviated to some extent from the one described. Thus, no clear initial "peak response" was seen, but blood flow tended to directly attain an approximately steady state level.

The quantitative relation between the venous hyperosmolality and the concomitant hyperemia response was analysed in these short term hypertonic infusion experiments with regard to changes evoked at the peak response and in the early phase of the steady state response. In the individual animal, repetitive infusions were usually performed, care being taken that full recovery of the vascular response was established in between consecutive infusions. A correlation was found between the evoked changes of vascular conductance and of venous osmolality both during the peak response and during the steady state response. This is exemplified by the experiment shown in Fig. 6 in which sodium lactate was infused at three different rates. During the peak flow responses, vascular conduc-

tance increased by 253, 340, and 560 per cent and venous osmolality by 9, 14, and 35 mOsm; the corresponding figures during the steady state responses were 74, 117, and 297 per cent and 15, 23, and 43 mOsm, respectively. In both cases there is a close correlation between the vascular and the osmolar changes. It may be pointed out that the peak response shown in the last panel of Fig. 6 represented a virtually maximal resistance vessel dilatation (flow increased from about 11 to about 60 ml/min $\times$ 100 g) as checked by a later supramaximal acetylcholine injection.

The bulk of data on osmolar changes during hypertonic infusion was obtained during the steady state vascular response ($n = 160$). In 61 of these infusions, observations during the peak response were also made. The collected data showed a correlation between the evoked increase of vascular conductance and the concomitant venous hyperosmolality both at the peak response ($r = 0.54$, $p < 0.001$) and at the steady state response ($r = 0.70$, $p < 0.001$). During the peak response, a maximal dilatation of the resistance vessels (checked by acetylcholine injection) was not infrequently present when venous osmolality was increased by 20 to 30 mOsm. At this level of venous hyperosmolality, there was usually some recovery of vascular tone after the peak response (steady state response), but at higher osmolality levels (40 to 100 mOsm) such recovery was often virtually absent indicating a maintained approximately maximal dilatation of the resistance vessels.

The question whether the dilator potency was different for the tested substances was analysed only for glucose and sodium lactate and only with regard to the steady state response. These substances were the ones tested in by far the largest number of experiments and the steady state observations provided a sufficiently large material for such an analysis. In the experiments in which vascular tone in the control period could be considered normal (see section A 3), linear regression analysis relating the evoked per cent increase of vascular conductance to the concomitant venous hyperosmolality gave the following equations for glucose and sodium lactate respectively: $y = 4.1x + 7.7$ ($n = 81$; $r = 0.80$; $p < 0.001$) and $y = 5.9x - 11.2$ ($n = 40$; $r = 0.88$; $p < 0.001$). Statistical analysis, in which the differences of the regression coefficients and of the intersects with the vertical axis were considered simultaneously (Scheffe's method, *cf.* Miller 1966), showed that above a venous hyperosmolality of 28 mOsm the sodium lactate dilator effect was significantly ($p < 0.05$) greater than the glucose effect.

During constant pressure perfusion, blood flow in the postinfusion period, like after exercise, usually showed an exponential decline. Thus, a strictly mono-exponential decay was present in 29 out of 44 semi-logarithmic plots of excess flow against time. The mean half-time ($T_{1/2}$) for the return of blood flow to the control level was for these 29 infusion experiments $86 (\pm 15 \text{ SEM})$ sec. This value is not different ($p > 0.05$) from the corresponding one calculated for the post-exercise period (A 1) suggesting that the rate, and perhaps the mode, of elimination of the dilator factors after exercise and after hypertonic infusion

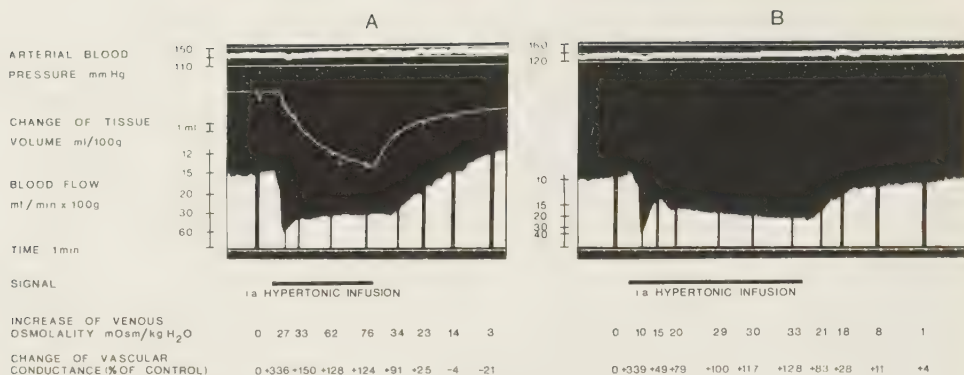


Fig. 7. Patterns of blood flow response during "prolonged" hypertonic infusion to resting skeletal muscle. Panel A (glucose infusion) shows the most commonly observed flow pattern, an initial peak and then a less pronounced but maintained hyperemic level despite a gradual rise of venous osmolality. Net transcapillary osmotic fluid absorption (tissue volume decrease) caused by the hypertonic infusion also shown. Panel B (sodium lactate infusion) illustrates how, after the initial partial recovery from the peak flow response, blood flow sometimes increased parallel to the increasing venous hyperosmolality.

were roughly similar (*cf.* Dornhorst and Whelan 1953, Mellander and Johansson 1968). Like in exercise, $T_{1/2}$ was not correlated to the degree of the preceding vasodilatation of the resistance vessels.

Fig. 7 illustrates changes of vascular conductance and venous osmolality during more prolonged (7–10 min) infusion periods. Panel A depicts the most commonly observed pattern of response: After the peak response, flow (and vascular conductance) remained at approximately constant levels throughout the infusion period, whereas venous plasma osmolality gradually increased with time. Sometimes (Panel B) the partial return of blood flow after the peak response was followed by a successive flow increase parallel to the rising osmolality. After cessation of the infusions, blood flow and venous osmolality in both cases gradually returned towards the control levels. Panel A also shows the changes of tissue volume caused by hypertonic infusion reflecting net transcapillary fluxes of fluid. During the infusion, an osmotic withdrawal of extravascular fluid to the intravascular compartment occurred and this fluid volume was drained from the region *via* the venous system. It can be seen that the rate of fluid absorption was most rapid in the initial phase of the infusion and then tended to decline with time. Upon cessation of the infusion, fluid gradually returned to the tissue. The extent to which the fluid absorption during hypertonic infusion influenced the calculations of the resistance changes will be considered below. It should finally be mentioned that prolonged hypertonic infusion sometimes induced an oscillatory flow pattern with minor fluctuations around a clearly increased mean flow level.

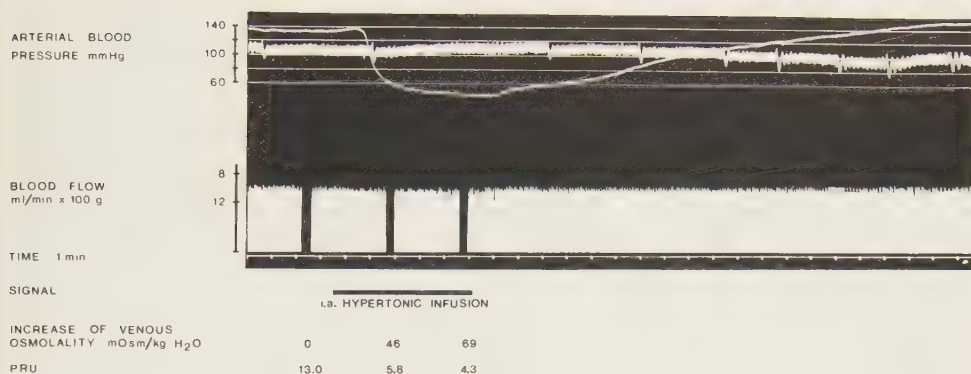


Fig. 8. Typical pattern of vascular resistance change in response to intra-arterial hypertonic infusion to resting muscle perfused at constant blood flow. Arterial blood pressure (resistance) showed an initial rapid decrease followed by a slower decline towards a steady state level. Note that there was no partial recovery of the hyperosmolar dilator response during constant flow perfusion.

The changes of vascular resistance caused by i.a. hypertonic infusion were studied in 55 constant flow perfusion experiments. The resistance effects were mainly observed after glucose and sodium lactate infusions and they were quite similar, whereas some experiments with urea showed a different response (see below). Fig. 8 illustrates the typical changes of resistance (perfusion pressure) observed with the two first-mentioned substances. It can be seen that hypertonic infusion (glucose) led to a rapid fall of arterial perfusion pressure within the first minute, after which a further, more gradual, decline followed. The initial rapid pressure drop corresponded to a regional vascular resistance decrease down to 45 per cent of control resistance which was 13.0 mm Hg/(ml/min x 100 g tissue). The concomitant rise of venous osmolality was 46 mOsm. The venous hyperosmolality increased further to 69 mOsm during the later phase of infusion where resistance had fallen to 4.3 mm Hg/(ml/min x 100 g), *i.e.* to 33 per cent of the control value. This implied a virtually maximal vasodilatation since a later administered supramaximal dose of acetylcholine decreased resistance to 3.8 mm Hg/(ml/min x 100 g). After cessation of the hypertonic infusion, perfusion pressure slowly increased towards the control level which was reached within some 20 min. The control infusion of isotonic glucose solution in this experiment lowered vascular resistance by only 4 per cent indicating that the main resistance effect described was caused by the hyperosmolality *per se*.

It should be noted that the resistance response to experimental hypertonicity evoked during constant flow perfusion diverged from the typical response during constant pressure perfusion insofar as the recovery of vascular tone (steady state response) observed with the latter technique was not present. This discrepancy will be considered below (Comments).

The resistance response induced by i.a. infusion of hypertonic urea solution differed from that described for the other solutes. During constant pressure perfusion a rapid and sometimes pronounced increase in blood flow was seen in the initial phase of the infusion but, despite continued infusion, flow then returned slowly towards the control level while exhibiting pronounced oscillations. When the infusion was stopped, the resistance vessels constricted further, and quite rapidly, as shown by the fact that blood flow was considerably less than in the control period before the infusion. Some 2 min later flow increased again to the control level. During constant flow perfusion hypertonic urea infusion evoked a similar transient dilator response but the oscillatory phenomenon and the post-infusion constriction were less pronounced.

Critical evaluation of the blood flow effects observed during hypertonic infusion. The fluid volume absorbed from the lower leg muscles during hypertonic infusion (*cf.* Fig. 7, Panel A) deserves consideration when calculating resistance effects since it forms part of the venous effluent. In the experiments performed with constant pressure perfusion, blood flow was recorded on the venous side and hence the fluid absorption implies that postcapillary flow has been correctly estimated whereas precapillary flow has been somewhat overestimated, leading, in turn, to an overestimation of the dilator response. The degree of fluid absorption varied with the induced change of osmolality and was also, as may be expected, related to the evoked hyperemia (*cf.* Chapter IV). Further, it was observed that the change of tissue volume was less pronounced during urea infusion than during infusion of the other solutes which in all probability can be explained by differences in the magnitude of the transcapillary osmotic reflection coefficient for the various solutes. The extent to which venous flow exceeds arterial flow can be determined from the recorded change of tissue volume. In the experiment depicted in Fig. 7 (glucose infusion), for example, the fluid absorption amounted to $5.0 \text{ ml/min} \times 100 \text{ g}$ during the peak blood flow, *i.e.* to about 8 per cent of the total venous effluent, and to $2.4 \text{ ml/min} \times 100 \text{ g}$ during the early phase of the "steady state flow period", *i.e.* to about 5 per cent of the total venous outflow. Similar calculations were made from recordings in 66 infusion periods in which the different solutes, except urea, were used. A considerable spread of data was found but mean values of the fluid absorption amounted to $11 (\pm 4.7 \text{ SD})$ per cent and to $7 (\pm 3.4 \text{ SD})$ per cent of the concomitant venous outflow during the peak response and in the early phase of the steady state flow response respectively. When relating the fluid absorption to the evoked *increase* of venous blood flow, the corresponding figures were $19 (\pm 8.8)$ per cent and $15 (\pm 8.3)$ per cent. To assess the overestimation of the resistance effect of experimental hyperosmolality, when judged from the evoked changes of venous blood flow only, the pre-/postcapillary resistance ratio during infusion also has to be known. Assuming this ratio, as an approximate mean value, to be 3:1 the recorded outflow values during the peak response should be corrected by 8 per cent and during the steady state response by 5 per

cent whereas the calculated per cent increase in vascular conductance should be corrected by 14 per cent and 11 per cent respectively. Such corrections, it should be stressed, have been made in the diagrams below, depicting the dilator effect of experimental hyperosmolality (Figs. 9 and 10). During constant flow perfusion the drop chamber generally was placed on the arterial side and consequently, precapillary flow was correctly obtained whereas the fluid absorption during hypertonic infusion implied that postcapillary flow was somewhat underestimated. Therefore total vascular resistance during infusion, in turn, has been overestimated to some extent, *i.e.* the effect of experimental hyperosmolality has been somewhat underestimated. Since, however, the major part of total vascular resistance is located in the arterial vessels and since the blood flow in these vessels was measured correctly, the error during constant flow perfusion will be small and has not been corrected for below (Fig. 11).

Possible rheological effects of the absorbed extravascular fluid during hypertonic infusion in capillary and postcapillary vessels were considered indirectly by intra-arterial infusions of isotonic solutions at similar rates. At the higher rates of infusion some increases of blood flow were noted. Since these effects were comparatively small and since the *i.a.* infusions affected the blood also on the arterial side of the vascular bed it can be concluded that rheological effects of transcapillary fluid absorption must have been quite insignificant.

During exercise the action of hyperosmolality on the vascular smooth muscle evidently is exerted from the extravascular (interstitial) space. This also seems to be the case during experimental hyperosmolality caused by *i.a.* hypertonic infusion as indicated by the experiments to be described in Chapter VI. It follows that if the barrier between the interstitial space and capillary blood prevents the establishment of an osmolar equilibrium during short-term hypertonic infusion, the extent of extravascular hyperosmolality will be overestimated when determined from samples of the venous effluent. To test whether venous osmolality exceeded the extravascular osmolality during hypertonic infusion the following experiments were designed. The muscle was perfused from a reservoir of arterial blood kept at a constant hyperosmolar level. Repetitive venous blood samples were withdrawn in the phase of induced hyperemia and in a second phase when blood flow was mechanically reduced to about 1/10 of that during the hyperemia response. The samples from the phase when capillary flow velocity was thus reduced, and hence favouring the capillary osmolar exchange, showed significantly lower osmolality values than those taken during the hyperemia phase. This was found in all infusion experiments of the type just described ($n = 12$) regardless of the extent of the initially produced hyperemia. The hyperosmolality of the venous samples withdrawn during reduced flow averaged 74 per cent of that during the hyperemia phase which strongly indicates that on osmolar equilibrium between capillary blood and tissue is not established during short-term hypertonic infusion experiments. Since, as mentioned, the interstitial hyperosmolality in all probability is the prime determinant

of the vascular response, the described experiments suggest that the dilator effect of experimental hyperosmolality may be considerably underestimated when related to the osmolar changes in the venous effluent. Such an effect of incomplete transcapillary osmolar equilibrium during hypertonic infusion has not been corrected for. Therefore, the dilator effects to be described (Figs. 9 and 10) may actually have been caused by osmolar changes in the interstitial space that were some 25 per cent lower than in the venous blood.

The described flow increases of hypertonic infusion might possibly, to some extent, be due to effects other than direct inhibition of vascular tone on an osmotic basis. For instance, hypertonic infusion might cause an osmotic dehydration of the vascular walls (which theoretically might increase the vascular lumina), lead to rheological effects by osmotic shrinkage of the blood cells, or cause indirect dilator effects *via* release of dilator agents in the blood or from remote organs (e.g. the adrenals). These possibilities seem to be refuted by the following experiments.

In five animals the vascular bed was dilated maximally by i.a. continuous infusion of a supramaximal dose of papaverine. An isotonic infusion, later shifted to a hypertonic infusion of the same rate, was then superimposed, the latter raising venous osmolality to levels exceeding the maximal ones produced in the experiments described above (>75 mOsm). In none of these experiments ($n = 10$) did venous outflow during hypertonicity increase by more than what could be explained by the concomitantly observed net transcapillary fluid absorption (mean increase being 7 per cent). These findings indicate that neither dehydration of the vascular walls nor shrinkage of the blood cells were phenomena of significance for the described hyperemia effects of experimental hyperosmolality.

The following type of experiment served to illustrate that hypertonicity in all probability did not cause a release or production of dilator agents in the blood itself. Samples of blood were made hypertonic up to a level of 700–800 mOsm by the addition of glucose implying that the hyperosmolality considerably exceeded that produced in the hypertonic infusion experiments. Plasma obtained from such pretreated samples was, after dilution to isotonicity, infused intra-arterially to the muscles but failed to induce a vasodilatation. It should be stressed in this connection that no macroscopic sign of hemolysis was ever noted in the plasma samples withdrawn in the hypertonic infusion experiments.

β -receptor blockade of the vascular bed (Propranolol) did not alter the magnitude of the hyperemia effects of experimental hyperosmolality. Apparently, hypertonicity did not cause a local β -receptor stimulation nor could the effects be ascribed to possible release of adrenaline from the adrenals; the latter conclusion was corroborated by experiments in which both these glands were ligated. The fact that the flow effects of hypertonic infusion was fully developed within a time period usually shorter than that required for recirculation indicated that they were not caused by dilator factors released from re-

mote organs. The described flow effects were not altered by dibenzyline or atropine treatment showing that hypertonicity did not interfere with local α -adrenergic or cholinergic mechanisms in the present experiments.

Although unlikely, the possibility exists that the observed flow effects to hypertonic infusion to some slight extent could be related to a change of pH since the infused solutions were not buffered to the pH of blood prevailing in the control period. However, any such influence was indirectly taken into account *via* the control isotonic infusions. Furthermore, measurements of venous blood pH during hypertonic infusions at high rates (glucose or sodium lactate) showed that only small and inconsistent changes were produced. On the average, venous blood pH in these experiments ($n=8$) was decreased by 0.007 units during the peak flow response and by 0.013 units after 5 min infusion.

The flow pattern in terms of a peak and a steady state response, observed during experimental hyperosmolality at constant pressure perfusion, indicates that there is some recovery of vascular tone in the latter phase. The phenomenon could only to a minor extent be ascribed to different rates of fluid absorption in the two phases of the response and was furthermore present in experiments in which muscle blood flow was recorded on the arterial side. A possible explanation for this phenomenon will be given in Chapter III, *i.e.* that it is caused by a myogenic constrictor reaction, evoked by increased transmural pressure in the small resistance vessels, which counteracts the dilator response in the steady state flow phase. Here, mention should only be made that some findings in the present series of experiment supported this hypothesis. Thus, it was shown that if the stimulus for such a myogenic reaction, *i.e.* an increase of small artery pressure during hypertonic infusion, was reduced by mechanical lowering of arterial inflow pressure, the distinction between a peak and a steady state response usually waned significantly or even disappeared. Furthermore, recovery of vascular tone tended to disappear when the muscle was perfused with cold (28°C) blood, a procedure considered to lessen or abolish myogenic reactivity (*cf.* Lundvall *et al.* 1967).

Another explanation for the peak to steady state flow pattern during hypertonic infusion could be that the peak effect was partially related to a transintimal dilator effect on the arterial side as a consequence of an especially high arterial blood osmolality in the earliest phase of the infusion, *i.e.* before blood flow increases. This possibility, however, seemed refuted by the experiments described in Chapter VI.

3.3. Comparison between dilator effects in resistance vessels evoked by exercise and by experimental hyperosmolality

In a comparative analysis of vascular effects evoked by different experimental procedures, *e.g.* exercise and experimental hyperosmolality, it is important that the control vascular tone is similar in both series of experiments, especially if the effects are expressed in per cent of the control values. This may be illustrated

by the following example: At equal perfusion pressure, one dilator factor may be found to increase blood flow in the sympathectomized skeletal muscle from, say, 10 to 40 ml/min $\times$ 100 g, *i.e.* by 300 per cent, and another from 4 to 40 ml/min $\times$ 100 g, *i.e.* by 900 per cent. The marked difference in the per cent flow changes may not necessarily represent a corresponding true difference in dilator potency.

Further, such a comparative analysis might be influenced by the state of the preparation from which the results are obtained. It cannot be entirely avoided that the anaesthesia, surgery, and instrumentation required for detailed circulatory studies on experimental animals sometimes cause some deterioration of the preparation. In consequence, an abnormally low vascular resistance may prevail due to partial loss of normal basal vascular tone or, conversely, an abnormally raised resistance, caused, for instance, by aggregation of the blood cells. Such signs were occasionally noted also in the present series of experiments. Aggregation, for instance, seemed present in some experiments of long duration and was revealed as a sudden and maintained decrease of a high resting vascular resistance upon a rapid *i.a.* injection of saline, apparently causing a washout of sludged cells. A similar washout effect evidently could be caused by exercise and hypertonic infusion under these circumstances, since much larger resistance responses were evoked before than after such a saline injection. Results obtained from deteriorating preparations may thus partially be due to artefacts and should be excluded, if possible.

In an attempt to minimize such possible errors in the quantitative evaluation of the present results, those experiments were excluded which on a statistical basis showed extreme values for control vascular resistance (conductance). For the constant pressure perfusion experiments, the mean value of vascular *conductance* for all control observations ($n = 214$) before exercise and hypertonic infusion was calculated and for the constant flow perfusion experiments the mean value of vascular *resistance* for all control observations ($n = 83$) was calculated; (note that the number of control observations before exercise was less than the number of experimental observations since graded work often was produced by step-wise increase of the sciatic nerve stimulation rate). Only those experiments were accepted in which control conductance (resistance) did not deviate from the mean value by more than ± 1 SD. In the first-mentioned series of experiments the mean control vascular conductance was 0.085 ± 0.032 SD (ml/min $\times$ 100 g)/mm Hg. For the constant flow experiments, the mean control vascular resistance was 16.0 ± 7.4 mm Hg/(ml/min $\times$ 100 g).

By this processing of the material vascular tone in all the remaining experiments can be considered normal or close to normal for the sympathectomized muscle (see Comments). It implied that 83 out of 104 exercise experiments and 129 out of 160 infusion experiments could be accepted in the series with constant pressure perfusion. For constant flow perfusion, the corresponding figures were 45 out of 59 exercise experiments and 40 out of 55 infusion experiments.

values evoked by exercise (open circles) and by experimental hyperosmolality (peak response, triangles; steady state response, closed circles), are plotted against the concomitantly observed increases of venous plasma osmolality. The diagram shows classed mean values ($\pm$ SEM) for ± 1.5 mOsm ranges. It should be pointed out that for all three series of experiments mean control vascular conductance was almost the same (0.083, 0.086, 0.080 (ml/min $\times$ 100 g)/mm Hg, respectively). For all three groups of data there was a correlation between the increase of vascular conductance and the degree of venous hyperosmolality ($r = 0.56$, $p < 0.001$ for the exercise data; $r = 0.61$, $p < 0.001$ for the peak response data; $r = 0.81$, $p < 0.001$ for the steady state response data). The conductance increase during exercise was about maximal ($\approx 380\%$) at a venous hyperosmolality of about 25 mOsm. The effects during the peak response of experimental hyperosmolality were quite pronounced and often tended to approach those during exercise. On the other hand, with regard to the steady state response, the effects were significantly smaller. At a venous osmolality increase of 40 mOsm, *i.e.* the upper level during exercise, the conductance increase during the steady state response was approximately 200 per cent. At a still higher osmolality (40—50 mOsm) the conductance changes during the peak response was about equal to the maximal ones during exercise. For the steady state response such pronounced effects were present above 60 mOsm. These latter findings deserve attention in view of the observation that a transcapillary osmolar equilibrium did not seem present during hypertonic infusion (section A 2).

The changes in blood flow evoked by exercise and experimental hyperosmolality are shown in Fig. 10 and the data are classed as in the previous figure. Since there was some variation in the perfusion gradient between the individual experiments the comparison of blood flows becomes relevant only if the pressure/flow relationship is taken into account. The average arterial blood pressure in the control period was 108 ± 1.5 SEM mm Hg and venous outflow pressure 5—8 mm Hg, thus giving a mean perfusion pressure close to 100 mm Hg. The average perfusion pressure during exercise and hypertonic infusion was about the same. In an attempt to compensate for pressure effects, all blood flow values have been converted to an arterial pressure of 108 mm Hg, assuming a linear pressure/flow relationship over the actual pressure range encountered. That this assumption was approximately correct was indicated by direct observations in individual experiments of the changes in flow that were elicited by mechanical adjustment of arterial pressure. It can be seen that the relations between the exercise hyperemia and the flow effects during the peak and during the steady state response of experimental hyperosmolality were similar to those in Fig. 9. Maximal mean blood flows, about 40 ml/min $\times$ 100 g at a perfusion pressure of 100 mm Hg, were reached at a venous hyperosmolality of about 25 mOsm during exercise and of 35—45 mOsm during the peak response. During the steady state response the hyperemia was not too far from maximum above 60 mOsm. A mean blood flow of about 40 ml/min $\times$ 100 g is a reasonable average figure for ma-

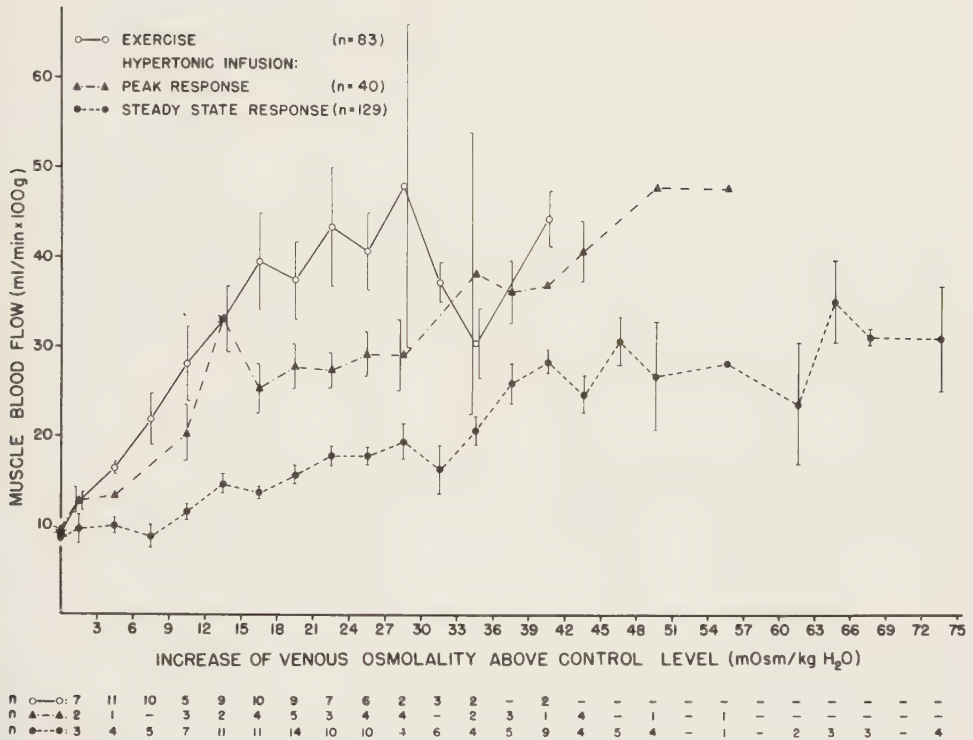


Fig. 10. Diagram showing blood flow in skeletal muscle (ml/min $\times$ 100 g at a perfusion pressure of 100 mm Hg) during exercise and during "experimental hyperosmolality" in resting muscle against the concomitant increase of venous osmolality. Data obtained from same experiments as shown in Fig. 9.

ximal blood flow during heavy exercise considering the biological variation (see below). It may be mentioned, however, that flow values exceeding 50 ml/min $\times$ 100 g were not infrequently observed both during work and experimental hyperosmolality.

Fig. 11 shows the resistance responses evoked by exercise (open circles) and by hypertonic infusion (closed circles) during constant flow perfusion. Vascular resistance during exercise and during hypertonic infusion is expressed in per cent of the control resistance (100 %) and plotted against the concomitant increase of venous osmolality. Mean control resistance was approximately equal in both series of experiments (exercise 17.1, experimental hyperosmolality 16.4 mm Hg/(ml/min $\times$ 100 g)). The diagram shows classed mean values ($\pm$ SEM) for $\pm$ 2.5 mOsm ranges. The data during exercise were obtained at a time period after the commencement of work (2 to 3 min) when the vascular and osmolar changes were approximately fully developed. The data during experimental hyperosmolality were obtained when the initial rapid vasodilator response was fully

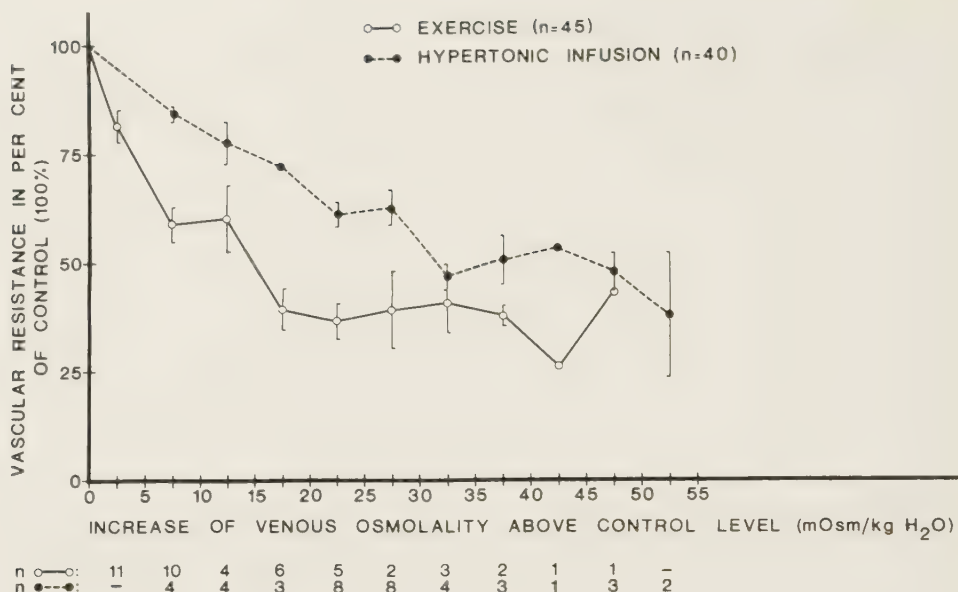


Fig. 11. Diagram showing, for constant flow experiments, vascular resistance in skeletal muscle, expressed in per cent of control resistance (100 %), during exercise and "experimental hyperosmolality" in resting muscle against the concomitant increase of venous osmolality above control level. Mean values $\pm$ SEM for ± 2.5 mOsm/kg H₂O ranges shown. Number of observations in each class shown at bottom of the figure.

established (*cf.* Fig. 8). It can be seen that the dilatations elicited by both exercise and experimental hyperosmolality were related to the degree of venous hyperosmolality ($r = -0.70$, $p < 0.001$; $r = -0.75$, $p < 0.001$). In the lower range of venous hyperosmolality (< 30 mOsm) the dilatations elicited by exercise were clearly more pronounced than those evoked by hypertonic infusion. Above this hyperosmolality level, the response to experimental hyperosmolality approached the exercise effect.

B. Precapillary "sphincters"

The influence of exercise and of experimental hyperosmolality on the precapillary sphincters was studied by following evoked changes in the capillary filtration coefficient (CFC) which reflect alterations in the capillary surface area available for exchange, *i.e.* in the number of patent capillaries (see Methods and Comments). CFC was determined by increasing venous outflow pressure by a known amount, hence raising the capillary hydrostatic pressure. It was calculated from the observed continuous increase of tissue volume persisting in the phase where the initial passive distension of the capacitance vessels was fully developed (see Methods). The CFC determination is complicated to some extent by

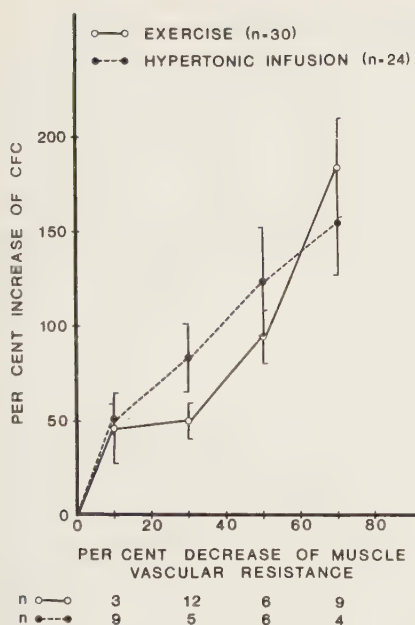


Fig. 12. Diagram showing per cent increase of CFC above control level in the constant flow perfused skeletal muscle during exercise ($n=30$) and during "experimental hyperosmolality" in resting muscle ($n=24$) against the concomitantly observed per cent decrease of vascular resistance below control level. The diagram shows classed mean values $\pm$ SEM. Number of observations in each class shown at bottom of the figure.

the fact that hypertonic infusion and exercise as such evoke net transcapillary fluid movements, mainly by osmosis (section A 1 and A 2 above and Chapter IV). Changes of blood flow will affect the rate of this osmotic fluid flux (Chapter IV), especially during hypertonic infusion because of the consequent changes in arterial blood osmolality (altered dilution of the infused solution in the blood). Therefore, the present analysis of changes in CFC was performed under constant flow perfusion. Even under constant perfusion the osmotic fluxes tend to change with time due to a gradually changing transcapillary osmotic gradient. This implies that the slope of the tissue volume curve before and after the period of raised venous outflow pressure is usually somewhat different. A reasonably correct estimation of CFC could, however, be made by calculating the induced fluid filtration from both these "control" slopes and by using the mean of the two values.

In 30 exercise experiments and in 24 hypertonic infusion experiments CFC was determined as soon as the resistance vessel dilatation was fully developed. Control vascular resistance could be considered normal in all these experiments according to the criteria mentioned in section A 3. Mean control CFC before exercise was $0.007 (\pm 0.0005 \text{ SEM})$ and before hypertonic infusion $0.010 (\pm 0.0005 \text{ SEM})$ ml/min $\times$ 100 g $\times$ mm Hg. CFC increased both during exercise and experimental hyperosmolality and roughly in proportion to the extent of the concomitant venous hyperosmolality.

The results are summarized in Fig 12 in which the observed increases of CFC are plotted against the concomitant changes in vascular resistance. The data are presented as the per cent increase of CFC above the control level versus the per

cent decrease of regional resistance below the control level (classed mean values $\pm$ SEM). There was a correlation between the two responses both during exercise ($p < 0.01$) and during experimental hyperosmolality ($p < 0.001$). It can be seen that the maximal increase of CFC averaged about 180 per cent during exercise and about 150 per cent during hypertonic infusion. These changes of CFC in all probability reflect corresponding increases of the perfused capillary surface area caused by relaxation of the precapillary "sphincters". Experimental hyperosmolality thus seems to influence this section of the vascular bed in similar way to exercise.

C. Capacitance vessels

Reactions within the capacitance vessels can in most situations be followed adequately by recording changes of tissue volume or by external monitoring variations in regional radioactivity of blood labelled with, for instance, ^{51}Cr , (e.g. Mellander 1960, Åblad and Mellander 1963, Kjellmer 1965 *a*). Determination of a capacitance response evoked by exercise or hypertonic infusion by recording of tissue volume is, however, made difficult because of the simultaneously evoked net transcapillary fluid fluxes which also contribute to volume changes. Instead, a more indirect approach was used. The vascular smooth muscle tone prevailing in the capacitance vessels in a steady state phase of exercise and of experimental hyperosmolality, respectively, was compared with that in a preceding control period. The extent of this tone in these different situations could be revealed by observing the acute increase of tissue volume that follows upon a short intra-arterial infusion of a supramaximal dose of acetylcholine known to be capable of bringing the capacitance vessels to virtually complete relaxation (e.g. Mellander 1966). It follows that the greater the response was to acetylcholine, the higher the prevailing tone of the capacitance vessels. Such acute increases of regional blood volume in response to acetylcholine could be recorded accurately by either of the above-mentioned volumetric and tracer methods and the simultaneous use of both these techniques in a pilot series of experiments showed that they both gave quite comparable results. In order to eliminate any significant effect of passive distension of the capacitance vessels resulting from a pressure rise during precapillary dilatation, all these observations were obtained during constant flow perfusion. The possible mechanical effect on the capacitance vessels of the muscle contractions *per se* during work was evaluated in addition by administering acetylcholine not only during the work but also in the immediate post-exercise period.

Each single experimental evaluation of the influence of exercise or experimental hyperosmolality on the tone of the capacitance vessels was thus based on a comparison of the effects of two acetylcholine infusions, one in the control situation and one during experimental intervention. The latter effect was expressed in per cent of the corresponding effect in the control situation. The present results are based on 16 such paired observations during exercise, 13 obser-

variations in the immediate post-exercise period, and 27 observations during hypertonic infusion. The volumetric method was used in most of these experiments but the ^{51}Cr -tracer method was applied in 7 experiments. In all the present experiments, control vascular resistance fell within a range considered normal (see section A3). The hypertonic infusion rates were adjusted so as to raise venous osmolality by at least 40 mOsm, *i.e.* to, or even above, the upper level encountered during work. Work intensity, in turn, was adjusted to elicit, on average, a resistance response similar to that during experimental hyperosmolality. Thus, vascular resistance (prior to the acetylcholine administration) averaged 55 per cent of control resistance during exercise, 52 per cent in the post-contraction period, and 45 per cent during hypertonic infusion.

The mean increase of regional blood volume (capacitance response) evoked by acetylcholine in the control situation was $0.20 (\pm 0.03 \text{ SEM})$ ml/100 g tissue in the exercise experiments and $0.26 (\pm 0.01 \text{ SEM})$ ml/100 g tissue in the infusion experiments. During exercise, in the post-exercise period, and during hypertonic infusion, the capacitance responses to acetylcholine were only slightly less, and averaged 80, 83, and 78 per cent of the control responses, respectively. There was no statistical difference between the three groups. These results indicate that both exercise and experimental hyperosmolality caused only little inhibition of the tone in the capacitance vessels despite the fact that the tone of the resistance vessels was markedly decreased. When scrutinizing the individual experiments it was found in each of the three groups that the magnitude of this inhibitory capacitance response tended to become greater the greater the dilatation of the resistance vessels, but even at a maximal resistance vessel dilatation the capacitance vessels were far from a state of complete relaxation. It may be noted that, although the main capacitance function is located on the venous side, a dilatation of the resistance vessels (mainly precapillary) leads to some increase of regional blood volume. The experimental data, when taken together, therefore suggest that the influence of exercise and experimental hyperosmolality on venous tone may be even smaller than that indicated by the above-mentioned figures for the decline of the capacitance response to acetylcholine. Because of the similarity between the results obtained during exercise and in the post-exercise period the conclusion could also be drawn that the muscle contractions caused no significant interference with the capacitance function at the rates of somatomotor fibre stimulation used.

COMMENTS

In this study, an attempt was made to examine the role of regional tissue hyperosmolality in exercise hyperemia by analysing on a quantitative comparative basis the reactions within the resistance vessels, precapillary sphincters and capacitance vessels in skeletal muscle (cat lower leg muscles) evoked by exercise and by experimental hyperosmolality, the latter produced by intra-arterial hypertonic infusion to the resting tissue. This investigation is a continuation and ex-

tension of a previous study (Mellander *et al.* 1967) and it confirms our previous conclusion that tissue hyperosmolality is a mediator in exercise hyperemia.

Pattern of response in consecutive vascular sections. In section A1, the characteristics of the changes in osmolality and vascular resistance were examined during graded exercise under both constant pressure and constant flow perfusion. Exercise was found always to be associated with locally produced tissue hyperosmolality, the extent of which was estimated to the amount it was reflected in the venous effluent. The hyperosmolality developed almost immediately at the commencement of work and within a few minutes reached a maximal level in the venous effluent. The dilator response of the resistance vessels showed a similar time course, commencing almost immediately upon exercise and reaching a maximal level within 1 to 2 minutes. The extent of venous hyperosmolality when fully developed showed a highly significant positive correlation to the magnitude of the concomitant dilator response of the resistance vessels, both these phenomena, in turn, being correlated to the intensity of the work (rate of somato-motor fibre stimulation, 0.25—4 imp/sec). During heavy (4 imp/sec) exercise venous osmolality could exceed that at rest by as much as 40 to 45 mOsm/kg H₂O. Venous hyperosmolality remained at the maximal level reached after 2—4 min throughout prolonged periods (15 min) of light and moderate exercise (0.25 to 2 imp/sec) but tended to decline during prolonged heavy work, and then parallel to a declining muscle tension.

When designing experiments aiming at disclosing the role of tissue hyperosmolality *per se* in exercise hyperemia, due attention must be paid to the fact that both the osmolar and vascular changes in work develop rapidly. Attempts were therefore made to create by i.a. hypertonic infusion an experimental hyperosmolality in the resting muscle which resembled as closely as possible that in exercise with regard to magnitude and time-course. The hypertonic solutions consisted of non-electrolytes or electrolytes, the latter usually sodium⁺lactate⁻since these two ions seemed mainly responsible for the interstitial hyperosmolality of exercise (A1). Since, as in the exercise experiments, changes in tissue osmolality during hypertonic infusion could not be determined directly, they were followed as they were reflected in the venous effluent. The results concerning the effects on vascular resistance of experimental hyperosmolality were presented in section A2. Experimental hyperosmolality was always associated with a rapidly developing dilator response of the resistance vessels which, in the constant pressure perfusion experiments, was shown as an initial large flow increase reaching a peak value within less than 50 sec usually followed by a steady state response at which flow stabilized at a somewhat lower level. There was a highly significant correlation between the magnitude of the dilator response of the resistance vessels (both the peak and the steady state response) and the concomitant increase of venous osmolality (Figs. 9 and 10). With regard to the peak response, a maximal dilatation of the resistance vessels was present at a rise of venous osmolality by 40—50 mOsm. To maintain an approximately maximal dilatation

as a steady state response, venous osmolality usually had to be raised by at least 60 mOsm above the control level. Similar conclusions were reached in the hypertonic infusion experiments performed under constant flow perfusion with the exception that there were no signs of a secondary partial recovery of resistance during the infusions. It was thus established that resistance and osmolar changes are correlated phenomena, both during exercise and during experimental hyperosmolality. Such a correlation was furthermore present during the phase of recovery of vascular tone in the post-exercise and post-infusion periods. The pattern of vascular response during exercise and experimental hyperosmolality was found to be similar not only with regard to the reactions in the resistance vessels but, as shown in section B and C, also in the precapillary "sphincters" (see further below) and in the capacitance vessels. Thus in both situations, the sphincters showed dilatations which increased gradually in relation to the evoked hyperosmolality, whereas the tone of the capacitance vessels was relatively little affected, if at all.

Analysis in the post-exercise period suggested that flow returned to the control level in an approximately exponential manner in consonance with the observation by Dornhorst and Whelan (1953). This seemed also true for the flow restoration after hypertonic infusion, and with a rate constant roughly similar to that after exercise. Dornhorst and Whelan concluded that if post-exercise hyperemia was related to the local concentration of some metabolite, its removal or destruction could not be critically dependent upon the rate of flow. It is therefore likely that elimination of the dilator factor(s) in exercise can depend upon several different processes. With regard to interstitial hyperosmolality it may be abolished after exercise *via* a wash-out to the blood stream, *via* re-uptake of osmolar products into the intra-cellular space of the striated muscle fibres, *via* water transfer into the interstitial space and *via* elimination with the lymph. It is possible that such modes of elimination lead to changes of interstitial hyperosmolality compatible with an exponential flow decay after exercise and also after experimental hyperosmolality.

A critical analysis was performed to reveal to what extent mechanisms other than true inhibition of vascular tone on an osmotic basis could have contributed to the changes of the resistance function noted in response to hypertonic infusion. First it should be stressed that rheological effects of the infused fluid *per se* and of the fluid absorbed from the extravascular space due to osmosis were taken into account in the presented diagrams, as well as the extent to which the latter phenomenon influenced the magnitude of the venous effluent. By special experiments the possible involvement of the following factors was analysed: 1. Hypertonic infusion might have caused a passive increase of vascular lumina by dehydration of the vascular walls or a passive resistance decline due to decreased viscosity of the blood as a result of osmotic shrinkage of the erythrocytes (*cf.* Overbeck *et al.* 1961); (the latter mechanism being somewhat remote in view of previous *in vitro* findings that the expected decrease in viscosity is counteracted

by a reduced viscous deformability of the osmotically shrunken red cells (e.g. Rand and Lacombe 1964, Meiselman *et al.* 1967, Schmid-Schönbein and Wells 1969)); 2. The hypertonic solutions could have caused release or production of dilator agents in the blood itself (for instance as a result of hemolysis, see Söderström 1944; however, Stainsby and Fregly (1968) reported pronounced vasodilation in muscle to hypertonic infusion when plasma was used as perfusate), or, 3. could have caused release of adrenaline from the adrenals or liberation of depressor agents from other tissues; 4. The response to hypertonic infusion could have been mediated *via* β -adrenergic or cholinergic mechanisms. The experimental evidence strongly suggested that none of these mechanisms could have played any significant role in the observed effects on the resistance function of hypertonic infusion in the present study. It may thus be concluded that the described changes in resistance vessels to experimental hyperosmolality most likely can be ascribed to a direct active inhibition of the vascular smooth muscle tone.

This opinion is strongly supported by a number of studies performed *in vitro* on a smooth muscle preparation, the rat portal vein, which shows functional characteristics similar to those of the single unit type of vascular smooth muscle residing in resistance vessels and precapillary sphincters (Mellander *et al.* 1967, Johansson and Ljung 1967, Johansson and Jonsson 1968, Arvill, Johansson and Jonsson 1969, Johansson 1969). These studies, considered in greater detail below (General discussion), showed that, owing to osmotic shrinkage of the effector cells, the spontaneous rhythmical electrical and mechanical activity of the vascular smooth muscle was promptly inhibited when the medium was made hypertonic and this inhibition was sustained when the increased osmolality was due to the addition of substances that did not readily penetrate the cell membranes of the vascular muscle (e.g. sucrose or glucose). Such an effect was not maintained if urea was used which is in consonance with the above described *in vivo* response to this particular substance which quickly penetrates the cell membranes.

The effects observed in precapillary sphincters and capacitance vessels deserve comment. Changes of precapillary sphincter activity were judged from observed alterations of CFC. CFC depends not only on the tone of the precapillary sphincters which, in turn, determine the number of patent capillaries and, hence, the capillary surface area available for filtration, but also on the specific permeability of the capillary membranes themselves. Any induced change of the latter factor will consequently influence the magnitude of the CFC. By studying the lymph/plasma ratio of dextran fractions of various molecular weights, Arturson and Kjellmer (1964) obtained strong evidence that exercise does not alter capillary permeability. Further, Landis and Sage (1971) found no evidence for increased capillary permeability when exposing mesenteric capillaries to pronounced hyperosmolality. The fact that CFC rose to similar magnitudes during pronounced experimental hyperosmolality and during heavy exercise in the present study can be taken as circumstantial evidence for unaltered capillary permeabi-

lity. It should then be noted that even a fairly small increase of capillary permeability during experimental hyperosmolality, if present, would be expected to have increased CFC clearly above the maximal value during exercise since transcapillary hydrodynamic flow varies with the fourth power of the capillary "pore" radius. It may be concluded that exercise and experimental hyperosmolality could dilate the precapillary sphincters to about the same extent.

The tone of the capacitance vessels was found to be insignificantly affected by exercise as well as by experimental hyperosmolality. With regard to exercise, the results confirm an earlier conclusion by Kjellmer (1964 *a*) who used a somewhat different experimental approach. The present reinvestigation of the capacitance response during exercise was prompted in order to study the possibility that the absence of a marked dilator response in the capacitance vessels might be due to a mechanical influence of the muscle contractions on the capacitance function which might have prevented them from dilating. The results obtained in the post-exercise period, however, made this explanation most unlikely.

It can thus be concluded that exercise and experimental hyperosmolality both can elicit the same differentiated pattern of vascular response in skeletal muscle: A pronounced dilatation within the resistance vessels and precapillary sphincters, no significant change of capillary permeability, and no significant active dilatation of the capacitance vessels. These findings support the view that tissue hyperosmolality can be a mediator of exercise hyperemia.

Some basic aspects of the approach to the comparative study of the resistance function. In a quantitative evaluation of the role of hyperosmolality in the exercise hyperemia response, emphasis, by definition, must be placed on the effects on the vascular resistance function. The techniques used to study this vascular function and the processing of the present data deserve consideration. The resistance function was analysed under constant pressure perfusion as well as under constant flow perfusion. Some advantages and some disadvantages are inherent in both techniques. The constant pressure perfusion method is, of course, the much more physiological one, since, as in the intact organism, blood flow is free to change. It provides quite accurate information about dilator responses over the entire range of changes in vascular smooth muscle tone, including states of maximal dilatation. In that respect the constant flow perfusion method is inferior, since, even if small to moderate dilator responses can be adequately followed, pronounced responses tend to become quantitatively underestimated. It is common knowledge, and also noted in the present study, that with this method vascular resistance in maximally, or close to maximally, dilated states hardly ever decreases to the same extent as under constant pressure perfusion. This may be due to abnormal influence of passive factors (e.g. passive elastic recoil, aggregation, or "critical closure") present under these somewhat unphysiological conditions which prevent perfusion pressure from declining to predicted very low levels. Exercise certainly can lead to a maximal resistance vessel dilatation, but

its full extent can thus not be revealed with this method. It follows that if a moderate dilator response to a test substance is quantitatively compared with the dilator effect of exercise, the relative potency of the test substance may be somewhat overestimated. Such an error may be inherent in several previous studies aimed at disclosing the roles of various candidates for exercise hyperemia and also in the present results obtained during constant flow perfusion. The bulk of data in this study was, however, obtained during constant pressure perfusion, and the quantitative evaluation will mainly be based on these experiments. Yet, the constant flow experiments formed a valuable complement to this study for reasons discussed below.

An obvious prerequisite for a correct quantitative evaluation of the vascular effects of any intra-arterially administered vasoactive substance is that the agent is distributed throughout the entire vascular tree to all parts of the studied region. This requires perfect mixing of the agent in the arterial blood, a demand difficult to satisfy during conventional intra-arterial infusion due to the prevailing laminar flow. In the constant flow experiments, the action of the perfusion pump was considered to cause efficient mixing, whereas in the constant pressure perfusion experiments this was shown to be achieved only if a special "mixing chamber" was inserted on the arterial side (see Methods) and such a device was used in all the present experiments of this type. That the dilator effects during hypertonic infusion would be seriously underestimated if thorough mixing was not attained was shown in a pilot study performed under experimental conditions identical to the present ones with the exception that no mixing chamber was used. A comparison of the results from that study (open circles) with the present ones (closed circles) can be seen in Fig. 13. For both series of experiments the steady state resistance responses to i.a. hypertonic glucose infusion, expressed as per cent increases of vascular conductance above the control level, are plotted against the concomitantly observed increase of venous plasma osmolality. Classified mean values for ± 2.5 mOsm ranges are given. The figure shows data obtained in experiments where control vascular conductance could be considered normal (see Section A3). Linear regression analysis was applied to the data (see regression lines). It can be seen that the evoked resistance responses were significantly greater with the mixing chamber than without it (sign test $p < 0.001$). This pronounced difference indicates that insufficient mixing is an important problem in conventional intra-arterial infusion studies and that this must be overcome to obtain reliable quantitative information. Special mention should be made that the mixing chamber as such caused no detectable interference with vascular tone. Thus, control resistance before and after the insertion of the chamber was very similar in all experiments indicating that the device caused no liberation of vasoactive substances in the blood and no cell aggregation. Further, vascular resistance was similar with and without the mixing chamber when in the resting muscle a threefold increase of blood flow was produced by a pump.

Local control mechanisms adjust the blood flow to exercising skeletal muscle

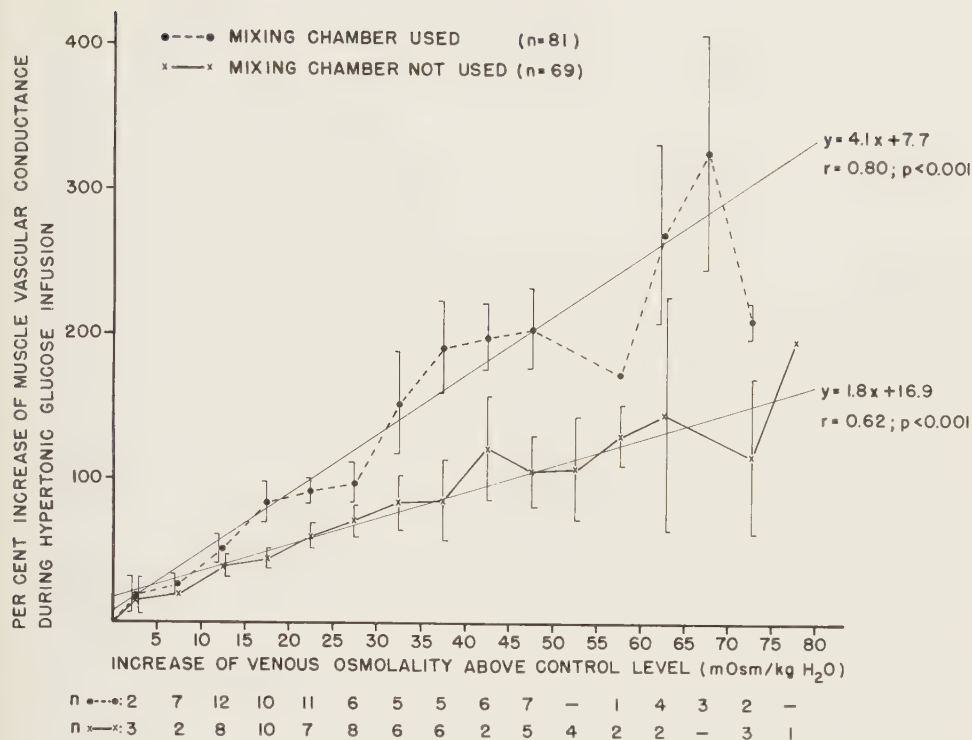


Fig. 13. Diagram showing the effect of the "mixing chamber" (see Fig. 1) on the magnitude of the resistance vessel dilator response to "experimental hyperosmolality" (glucose) in resting muscle. The per cent increase of vascular conductance is plotted against the concomitant venous hyperosmolality evoked with (broken line) and without (solid line) use of the mixing chamber. Data refer to the early "steady state" dilator response (mean values $\pm$ SEM for ± 2.5 mOsm/kg H₂O ranges). Number of observations in each class indicated. The hyperosmolar dilator response was much more pronounced when the mixing chamber was used (cf. regression coefficients), an effect ascribed to a more homogenous distribution of hypertonic solution in the muscle.

so as to satisfy, within limits, the tissue demand for a supply of nutrients and oxygen and for the removal of waste products. Muscle blood flow is linearly related to the work performed over a wide range of work intensities (e.g. Kramer *et al.* 1939, Wright and Sonnenschein 1965). At a given rate of motor nerve stimulation, the muscle contraction force decreases in relation to an enforced limitation of blood flow (Hirvonen and Sonnenschein 1962). Blood flow is thus of prime importance for skeletal muscle function. Investigations of mechanisms involved in exercise hyperemia should therefore preferably be made under constant pressure perfusion when flow is free to change. It would also seem most appropriate to express the dilator responses of the resistance vessels to exercise, or to a factor involved in this reaction, in terms of changes of blood flow, or of

vascular conductance (flow/pressure). Vascular effects are, however, often expressed as changes of resistance (pressure/flow). Due to the inverse relation of resistance to flow, which is the prime variable, there will be, simply for mathematical reasons, a successively poorer resolution of the resistance data the greater the vascular response which is not the case using flow or conductance. In a study like this one, in which the vascular effects of a possible mediator in exercise hyperemia are to be compared with those of exercise itself, this fact implies that the importance of the factor would appear considerably greater if based on resistance instead of conductance data, as illustrated by the following example. Suppose, for instance, that a constant pressure of 100 mm Hg, such a factor increased blood flow from 10 to 25 and exercise from 10 to 40 ml/min $\times$ 100 g tissue. This corresponds to a resistance change from 10 to 4 and from 10 to 2.5 units, respectively, and a conductance change from 0.10 to 0.25 and from 0.10 to 0.40 units, respectively. The factor might then be held responsible for 80 % of the exercise response if calculated from the resistance data, but only for 50 % if derived from the conductance data. This calculation may serve to illustrate the difficulty in evaluating results from different investigations unless due attention is paid to the presentation of the material in terms of resistance or conductance data. In the present study all data from the constant pressure perfusion experiments were expressed in terms of changes of conductance or flow. It should be stressed, however, that dilator responses obtained in experiments using constant flow perfusion may most appropriately be expressed in terms of resistance changes, since, under these circumstances, resistance varies directly with the recorded variable (perfusion pressure), and this convention was adopted in the present constant flow experiments.

In recent years it has been clearly established that the magnitude of a vascular response, *e.g.* a dilator response of the resistance vessels, to a given stimulus is significantly influenced by, and often directly related to the level of vascular tone prevailing in the control period prior to the application of the stimulus (*e.g.* Myers and Honig 1969). This phenomenon which was observed also in the present study may be related to different mechanisms. The vascular smooth muscle may vary its capacity to respond with its initial degree of stretch, determined for instance by the prevailing vascular transmural pressure, or through a possible effect on smooth muscle membrane potential (Myers and Honig 1969). It might also to some extent be related to more passive, mechanical effects, such as a washout of sludged cells as flow increases. Prior to the stimulus sludging could have created an abnormally high resistance. Whatever the case, it must be very important in any quantitative study of vascular effects, especially in a comparative investigation like the present one, to avoid in the evaluation of the results the influence of this phenomenon. In the present study this was done by basing the comparison of the vascular effects of exercise and experimental hyperosmolality on experiments in which the vascular tone prior to experimental intervention was equal (see section A3). Control vascular conductance averaged for

the whole material of constant pressure perfusion experiments $0.085 \text{ (ml/min} \times 100 \text{ g tissue)/mm Hg}$ which thus corresponds to an average blood flow of $8.5 \text{ ml/min} \times 100 \text{ g tissue}$ at a perfusion pressure of 100 mm Hg. These figures are consistent with those reported for other extensive materials concerning resting tone in the acutely sympathectomized white skeletal cat muscle (for ref. see Barcroft 1963, Mellander and Johansson 1968). This justifies the conclusion that resting vascular tone in the present experiments could be considered normal for the denervated muscle vascular bed. Even under these circumstances, there will of course be some spread of the results due to biological variation etc, which necessitates that the conclusions are based on a sufficiently large material. This demand is believed to be satisfied in the present study in which, with regard to resistance effects, the total number of experiments on preparations with normal resting vascular tone was 212, of which 83 refer to exercise experiments and 129 to hypertonic infusion experiments.

Quantitative evaluation of the role of tissue hyperosmolality in exercise hyperemia. The present results show that exercise is associated with a pronounced increase of tissue osmolality. This environmental change develops rapidly and in parallel to the functional hyperemia, and it reaches at a given work intensity a maximal level within a few minutes after commencement of exercise. To reveal the possible role of tissue hyperosmolality in exercise hyperemia it was considered important that the experiments in which experimental hyperosmolality was produced were designed so as to mimic as closely as possible the osmolar changes during exercise both with regard to magnitude and time-course. This could be done by acute relatively shortlasting hypertonic infusions and emphasis is therefore placed on the dilator responses of the resistance vessels evoked in the early period of the infusions. The diagrams in Fig. 9 and 10 summarized in quantitative terms the vascular conductance and blood flow increases evoked by exercise and experimental hyperosmolality in relation to the concomitantly observed increases of venous osmolality. Fig. 11 summarized the corresponding results for the constant flow perfusion experiments. Fig. 14, derived from Figs. 10 and 11, serves to illustrate the extent to which tissue hyperosmolality may contribute to the exercise hyperemia response. In this diagram the resistance vessel dilator responses during experimental hyperosmolality have been expressed in per cent of the exercise dilator responses at corresponding levels of venous hyperosmolality up to the highest ones encountered in exercise. Thus, for the constant pressure perfusion data (Fig. 10), the *increase* of blood flow above the control level during experimental hyperosmolality has been expressed in per cent of the *increase* of blood flow during exercise; for the constant flow data (Fig. 11), the *decrease* of vascular resistance (% of control) during experimental hyperosmolality has been expressed in per cent of the corresponding *decrease* evoked by exercise. At any level of venous hyperosmolality, the exercise hyperemia response is set to 100 per cent. To facilitate this deduction, the random variation in the results (Figs. 10 and 11) was neglected by drawing by eye a smooth curve for

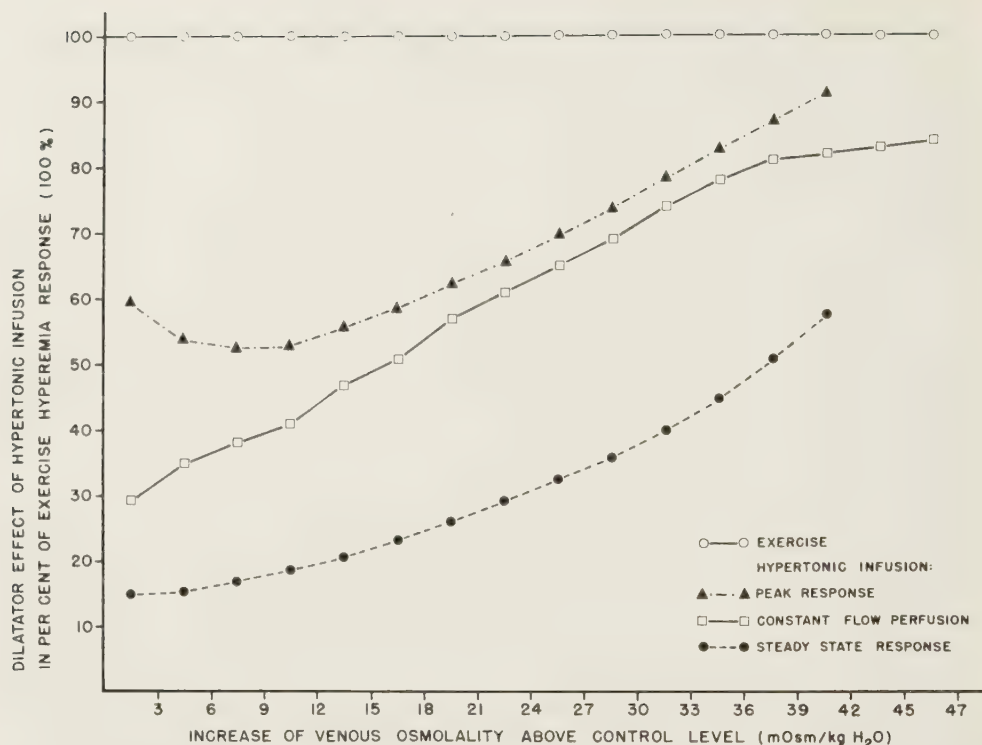


Fig. 14. Diagram showing the extent to which tissue hyperosmolality in working muscle may contribute to the exercise hyperemia response. The resistance vessel dilator response to "experimental hyperosmolality" in the resting muscle (blood flow increase above control level) is expressed in per cent of the exercise hyperemia response (blood flow increase above control level) at the given levels of observed venous hyperosmolality. The exercise hyperemia response at any level of work induced venous hyperosmolality is set at 100 per cent. The curves refer to the "peak" and "steady state" dilator effects of experimental hyperosmolality during constant pressure perfusion and to corresponding observations under constant flow perfusion (see key). Although different, all three curves suggest that tissue hyperosmolality is an important mediator of exercise hyperemia. Which one of the curves is most relevant in this respect is discussed.

each series of experiment taking into consideration the number of observations in each point. (When constructing the exercise hyperemia curve the fortuitous (?) low blood flow value between 33 and 36 mOsm (Fig. 10) was ignored).

It can be seen from Fig. 14 that for the constant pressure perfusion experiments the peak response during experimental hyperosmolality corresponded to some 50 to 90 per cent of the exercise hyperemia response. The corresponding fraction for the steady state response was considerably smaller varying from some 15 to some 60 per cent. The middle curve illustrates corresponding data for the constant flow perfusion experiments and it shows that the dilator effects

of experimental hyperosmolality under these conditions amounted to some 30 up to some 85 per cent of the exercise hyperemia response.

No doubt the curves in Fig. 14 are different and problems arise in deciding which one best depicts the quantitative role of tissue hyperosmolality in exercise hyperemia under these conditions of short-term exercise. It would seem that the effect can be no less than shown by the steady state response curve and no more than shown by the peak response curve. Common to all three curves is that they incline with increasing hyperosmolality. This can be taken to indicate that the relative role of tissue hyperosmolality in exercise hyperemia is greater the greater the work intensity, since, as shown in Fig. 4, the degree of work induced hyperosmolality was correlated to the rate of somato-motor fibre stimulation ("work intensity"). As mentioned above (A 2) and as will be discussed in Chapter III a counteracting myogenic constrictor effect may be responsible for the partial recovery of vascular tone upon transition from the peak to the steady state dilator response to hypertonic infusion. The stimulus for this myogenic reaction, *i.e.* a rise of transmural pressure in the smallest precapillary vessels, is not present during exercise nor during the early phase of the peak response and apparently neither during constant flow perfusion when the drop of perfusion pressure should tend to balance an increase of transmural pressure. The fact that the peak response curve and the constant flow curve in Fig. 14 are quite similar lends support to this view. The mentioned transmural pressure increase may be considered an experimental artefact gradually developing during hypertonic infusion (Chapter III). Circumstantial evidence therefore suggests that the steady state response curve of Fig. 14 may be less valid than the two others for the evaluation of the role of tissue hyperosmolality in exercise hyperemia. The peak response data and the constant flow data, when considered together with *average* values for tissue (venous) hyperosmolality induced by exercise (Fig. 4), suggest that more than 40 per cent of the exercise hyperemia can be ascribed to tissue hyperosmolality during light short-term exercise and more than 60 per cent during heavy short-term exercise. Whichever of the curves in Fig. 14 is used for the evaluation, however, the data strongly suggest that tissue hyperosmolality is an important mediator of functional hyperemia in muscle. This opinion is strengthened by the observation that a transcapillary osmolar equilibrium may not be present during exercise and hypertonic infusion (A 1, A 2, Chapter IV). Such a phenomenon, as mentioned (*e.g.* A 3), would lead to an underestimation of the role of hyperosmolality in exercise hyperemia with the present approach to the problem and it was not corrected for in the data of Fig. 14.

The data presented above (A 1) showed that tissue hyperosmolality was maintained during prolonged "light" and "moderate" work. During "heavy" work, however, it decreased with time but then in parallel with a drastic decline of the developed muscle force (Table I). Hypertonic infusions elicited dilator effects throughout prolonged infusion periods, but the dilator response was then usually maintained at a relatively steady level despite a gradual rise of osmo-

lality (Fig. 7). A myogenic constrictor mechanism, as discussed above, could possibly explain this maintenance of flow (vascular tone). Tissue hyperosmolality may thus be importance for the exercise vasodilatation not only in early but also in later phases of continuous work at least if it is not too strenuous. Such a maintained dilator effect would be present provided the substances responsible for the work induced tissue hyperosmolality do not readily penetrate into the vascular smooth muscle cells as will be commented upon in General Discussion. In that chapter studies from other laboratories concerning vascular effects of hypertonic infusion will also be considered.

CHAPTER III

CHANGES OF THE PRESSURE DROP CURVE AND OF RESISTANCE ALONG THE VASCULAR BED OF MUSCLE EVOKED BY EXERCISE AND "EXPERIMENTAL HYPEROSMOLALITY"

The results presented in the previous chapter showed that the patterns of vascular response to exercise and experimental hyperosmolality were quite similar with regard to the effects on the overall resistance function, on the precapillary sphincters, and on the capacitance vessels. These findings thus supported the hypothesis that tissue hyperosmolality produced by muscle work is a mediator of exercise hyperemia. The present study was undertaken to obtain a more detailed picture of the resistance function during exercise and experimental hyperosmolality, insofar that the evoked changes of resistance in delimited sections along the vascular tree were analysed. This was done by measuring blood pressure at various points from artery to vein as well as total muscle blood flow. The collected data permitted detailed evaluation of induced changes of the pressure drop curve in the vascular bed during exercise and during the so called "peak" and "steady state" dilator responses to hypertonic infusion. With the aid of these pressure curves, the resistance changes in very delimited parts of the vascular tree could be approximately revealed. This analysis suggested that the peak dilator response rather than the steady state response to hypertonic infusion may be most relevant for the quantitative evaluation of the role of tissue hyperosmolality in exercise hyperemia (*cf.* Fig. 14, Chapter II).

METHODS

Experiments were performed on 26 cats of both sexes weighing 2.8—5.0 kg. Observations were made during constant pressure perfusion on the acutely denervated vascular bed of the lower leg muscles. The anaesthesia and the main surgical procedures were the same as described in Chapter II. Venous outflow of blood from the studied region was recorded continuously by the technique described in that section. The arterial inflow to the muscles was diverted from the femoral to the popliteal artery through a short shunt circuit in which a "mixing chamber" was inserted to ensure thorough mixing during hypertonic infusion (Chapter II).

Arterial inflow pressure (AP), venous outflow pressure (VP), as well as pressure from a small intra-muscular arterial vessel (SAP) were measured in all experiments. In 9 of these, pressure was also monitored from a small venous vessel (SVP). AP and VP were measured close to the cannulated popliteal artery and vein from T-tubes. The cannulas inserted into these vessels were found to

cause no measurable pressure drop at any of the flow rates encountered. The SAP and SVP were obtained using in principle the method described by Haddy *et al.* (1954) implying that pressure in small vessels within the tissue is transmitted *via* collaterals to cannulated and proximally occluded vessels. For these measurements the sural artery and vein on the lateral surface of the gastrocnemius muscle were ligated and cannulated distal to the point of ligation with drawn-out flexible nylon tubings. Test experiments showed that an induced pressure change was transmitted with no measurable delay through these cannulas. By this cannulation technique SAP measurements were obtained, apparently largely at random, from fairly large down to small precapillary vessels (*cf.* Results section). To obtain more regularly pressure measurements from the smallest precapillary vessels the arterial cannula was instead inserted in a small branch of the sural artery and advanced along the branch into the muscles. In some experiments two SAP measurements were obtained simultaneously by using both these cannulation techniques. AP and SAP were recorded with Statham P 23 AC transducers and VP and SVP with Statham P 23 DC transducers connected to a Grass Polygraph. Sometimes VP was recorded with an Elema pressure transducer on an Elema Electrocardiograph (Model 81). The pressure recording systems (baseline, calibration) were tested frequently during the course of the experiments against standard pressure (saline columns). AP was recorded as the mean pressure via electrical integration. During exercise SVP and VP were also recorded as mean pressures to eliminate phasic pressure changes induced by the muscle contractions. The tubings between the places of pressure measurement and the pressure transducers were filled with heparinized saline solution and flushed at intervals during the experiments. A free communication between the small intramuscular vessels and the vessels cannulated for SAP and SVP measurements was shown in several ways. Quick drainage of blood was obtained when the small pressure cannulas were disconnected from the pressure transducers; further, SAP and SVP rapidly changed when the arterial inflow or venous outflow tubings were occluded, a test performed at intervals for a few seconds throughout each experiment. A few experiments in which free communication was not present were discarded.

Muscle exercise (single twitches) was produced by electrical stimulation of the distal end of the cut sciatic nerve using a Grass stimulator model S 4K. The stimulation characteristics (5 V, 0.5 msec, 0.25—4 imp/sec) were chosen so as to avoid any excitation of the sympathetic fibres (see Chapter II, Methods). Hypertonic (≈ 1500 mOsm/kg H_2O) solutions (usually glucose but sometimes sodium lactate) were infused into the arterial shunt at rates between 0.3 and 1.9 ml/min using a constant infusion apparatus.

Total vascular resistance (R_T) was calculated as $(AP - VP)/\text{flow}$. Resistance proximal (R_P) and distal (R_D) to the points in the vascular tree from where small vessel pressures were derived could be defined with regard to the SAP as well as with regard to the SVP measurement. Thus $R_P = (AP - SAP)/\text{flow}$;

or $\bar{R}_P = (AP - SVP)/\text{flow}$. $R_D = (SAP - VP)/\text{flow}$; or $R_D = (SVP - VP)/\text{flow}$. $(SAP - SVP)/\text{flow}$ gave small vessel resistance.

RESULTS

In the experiments to be reported mean arterial pressure at the place of the popliteal artery (AP) averaged 106 ± 1.7 SEM mm Hg and popliteal venous out-flow pressure (VP) 6 ± 0.2 SEM mm Hg in the resting control period before experimental intervention. Small artery pressure (SAP) in the control period varied between 88 and 13 mm Hg and small venous pressure (SVP) between 30 and 9 mm Hg. It should be pointed out that in the individual experiment control SAP almost invariably exceeded control SVP. In order to standardize the experimental conditions and make possible a valid comparison between results obtained during exercise and during hypertonic infusion, only those experiments were accepted in which resting control total vascular conductance (resistance) could be considered normal according to the criteria given in Chapter II, A3. This implied that control vascular conductance varied between 0.053 and 0.117 (ml/min $\times$ 100 g)/mm Hg. Under these standardized conditions the variation in the magnitude of the small pressures in the control state could be regarded as a sign that pressure measurements were obtained from distinctly different points along the vascular tree. The SAP (88 — 13 mm Hg) could thus be considered derived from proximal down to the smallest precapillary vessels. In between each experimental intervention a sufficient time period elapsed to allow the small vessel pressures and the vascular resistances (R_T , R_P , R_D) to return approximately to their control values.

A. Exercise experiments

Fig. 15 shows representative original tracings of blood flow and of the various pressures during graded exercise. In response to the step-wise rate of somatomotor fibre stimulation, blood flow increased from a control value of 6.3 ml/min $\times$ 100 g to 12.6 ml at 0.5 imp/sec, to 19.0 ml at 1 imp/sec, and to 62 ml/min $\times$ 100 g tissue at 4 imp/sec. AP was about 95 mm Hg in the control period and did not change much during the exercise whereas VP increased gradually from 6 up to 10 mm Hg. SAP was 56 mm Hg in the control period and it declined soon after the commencement of exercise to reach, within about one minute, a steady state level of about 31 mm Hg at 0.5 imp/sec. At 1 imp/sec there was some further pressure drop to 29 mm Hg while at 4 imp/sec SAP again rose to 33 mm Hg. SVP increased from 14 mm Hg to about 18, 20 and 28 mm Hg during the three exercise periods. Upon cessation of exercise, blood flow, VP, and SVP showed a gradual recovery to the control values, reached in about 15 min. SAP showed a slight further decline soon after work and then a slow return towards the control level. At the time when blood flow had returned to the control level, SAP was still some 15 mm Hg below the control value and it took another 15 min for complete recovery; (note that no significant *abrupt*

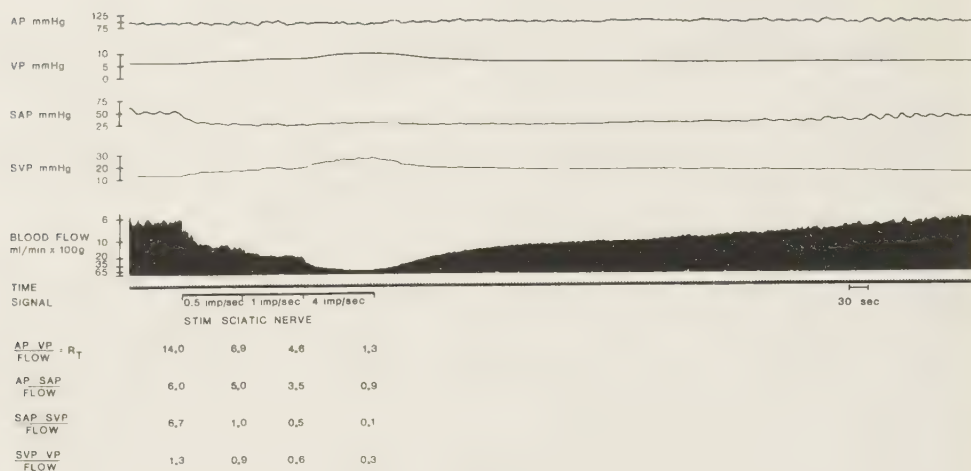


Fig. 15. Tracings of blood flow and of arterial inflow pressure (AP), venous outflow pressure (VP), small artery pressure (SAP), and small vein pressure (SVP) in skeletal muscle during graded exercise. Calculated values for total resistance (R_T) and for resistances in various segments of the vascular bed shown at bottom of the figure.

change in SAP, SVP or VP occurred in the immediate postcontraction period indicating that the single twitches *per se* did not contribute to the observed pressure changes during work).

The pressure in a small vessel at any moment is determined by the magnitude of AP and VP and by the relation between the vascular resistances distal (R_D) and proximal (R_P) to the point in the vascular tree from where the pressure is derived. At constant AP and VP, a relatively more pronounced decrease of distal resistance than of proximal resistance leads to a decrease of small vessel pressure, whereas, when the relative resistance changes are the opposite ones, small vessel pressure increases. R_D/R_P can be calculated for the points from which small artery as well as small vein pressures were measured. With regard to the SAP measurement in Fig. 15, R_D/R_P changed from a control value of 1.32 to 0.38 during exercise at 0.5 imp/sec, to 0.32 at 1/sec, and to 0.40 at 4/sec. The observed changes of SAP can be mainly ascribed to the changes of R_D/R_P , since AP and VP did not alter much. With regard to the point of SVP measurement, R_D/R_P increased progressively with increasing rate of somatomotor fibre stimulation. Information about changes of total and segmental vascular resistances in absolute terms can be obtained from the concomitant observations of the pressures and blood flow. These data for the described experiment are shown at the bottom of the record (Fig. 15). Special mention may be made to the fact that during light exercise (0.5/sec) the fall in R_T was accomplished mainly by a dilatation of the small vessels, see (SAP — SVP)/flow, whereas during heavy exercise (4/sec) the additional fall in R_T to a great extent

also depended upon relaxation of the larger resistance vessels situated proximal to the point of the SAP measurement, see $(AP - SAP)/\text{flow}$. Note further that, especially during heavy work, the pressure drop increased markedly in the proximal arterial and the distal venous segments, while the pressure drop across the small vessels was considerably reduced. At the time after exercise when blood flow and R_T had returned to the control levels, *i.e.* towards the end of the registration, SAP was still not restored. This was due to a redistribution of vascular resistance, insofar that proximal arterial resistance, $(AP - SAP)/\text{flow}$, was somewhat larger and small vessel resistance, $(SAP - SVP)/\text{flow}$, correspondingly smaller than in the control period at rest.

A relative estimation of the level in the vascular tree at which small vessel pressure is measured in this type of experiment can be obtained by calculating the ratio of R_P/R_T at resting vascular tone in the control period. This permits a functional division of the vascular tree with regard to resistance. The ratio approaches zero when "small vessel pressure" is derived from the most proximal arterial vessel and approaches unity when it is derived from the most distal venous vessel. A value of 0.80 to 0.85 may be taken to represent the capillary level, assuming a capillary hydrostatic pressure of 20 to 25 mm Hg at normal pressure head (see below).

In the experiment just described, R_P/R_T calculated for the SAP value in the control period was 0.43. Tables III and IV show representative data on the changes of pressure and resistance in response to graded exercise obtained in experiments where SAP was derived from a more proximal point (Table III) and a more distal point (Table IV) than in this experiment. Thus, R_P/R_T in the control period was 0.21 in the former experiment and 0.75 in the latter. The data in these two tables, like those of the experiment shown in Fig. 15, show that the main resistance decline during exercise occurs in the smaller precapillary vessels. It may be noted from Table III that, although R_T decreased during light (0.25 imp/sec) exercise, proximal arterial resistance in fact concomitantly increased above the control level by some 20 per cent. Such a resistance increase was a constant finding in experiments where the most proximal arterial segment was analysed. The resistance increase in this segment can possibly be ascribed to passive factors (see Comments). However, as work intensity increased (0.5, 1.0 and 4 imp/sec), this effect was overcome by a dilator influence of exercise. Note should also be taken in Table III of the very steep pressure drop across the proximal arterial segment and the concomitant small pressure drop across the vessels located distal to the point in the vascular tree from where SAP was derived.

From the experiment in Table IV a rough estimate of the changes of mean capillary pressure during exercise may be obtained, since SAP and SVP were apparently derived from points relatively close to the capillary level. Assuming that mean hydrostatic capillary pressure (P_c) in this experiment roughly can be taken as the mean of SAP and SVP, P_c in the control period would be 25 mm Hg, at 0.75 imp/sec 22 mm Hg, and at 4 imp/sec 29 mm Hg. These figures

TABLE III

	AP mm Hg	SAP mm Hg	VP mm Hg	Blood flow ml/min x 100 g	(AP-SAP)/ Flow = R _P mm Hg ml/min x 100 g	(SAP-VP)/ Flow = R _D mm Hg ml/min x 100 g	(AP-VP)/ Flow = R _T mm Hg ml/min x 100 g
Control	95	77	8	6.6	2.7	10.5	13.2
Exercise 0.25/sec	93	60	8	9.9	3.3	5.3	8.6
Exercise 0.5/sec	97	45	10	21.0	2.5	1.7	4.1
Exercise 1/sec	92	29	11	29.2	2.2	0.6	2.8
Exercise 4/sec	87	24	13	60.4	1.0	0.2	1.2

TABLE III. Arterial inflow pressure (AP), small artery pressure (SAP), venous outflow pressure (VP), blood flow, and calculated total resistance (R_T) and resistance proximal and distal to the site of SAP measurement during graded exercise. SAP in this experiment derived from a relatively proximal point in the vascular tree. For detailed description see text.

TABLE IV

	AP mm Hg	SAP mm Hg	SVP mm Hg	VP mm Hg	Blood flow ml/min x 100 g	(AP-SAP)/Flow mm Hg ml/min x 100 g	(SAP-SVP)/Flow mm Hg ml/min x 100 g	(AP-VP)/Flow mm Hg ml/min x 100 g
Control	115	33	16	6	10.0	8.2	1.7	10.9
Exercise 0.75/sec	110	23	21	8	15.4	5.7	0.1	6.6
Exercise 4/sec	100	29	28	10	37.7	1.9	0.0(3)	2.4

TABLE IV. Data from similar experiment to that shown in Table III. SAP derived from a relatively distal point in the vascular tree. For detailed description see text.

suggest that P_c is not changed very much by exercise, the effects of the rise in SVP to a great extent being balanced by the fall in SAP. It should be stressed, therefore, that an observed change of SVP *per se* cannot, as sometimes is done, be taken as an indicator of a corresponding change in P_c . The data also suggest that resistance and hence the pressure head across the capillaries can indeed be small during exercise.

From an analysis of the changes of segmental resistances during the development of the exercise hyperemia in the initial phase of graded exercise the following conclusions could be drawn. During light exercise there was a gradual dilator response reaching a steady state level within 1—2 min and this response preferentially was confined to the small vessels. During heavy exercise there was a more rapid dilator response which initially also mainly involved the small vessels. As R_T approached its low level, the more proximally situated arterial resistance vessels were also recruited in the dilator response. With regard to the post-exercise period, and especially after heavy and prolonged work, a regular observation was that SAP remained low for a considerable time after that R_T and blood flow had been restored to the control levels. Thus, while R_T was restored within 10 — 25 min, sometimes SAP did not return to the control level until 60 or more minutes after the cessation of the work. As mentioned above, maintenance of a low SAP value could be ascribed to a redistribution of vascular resistance, proximal arterial resistance being larger and small vessel resistance smaller than in the control period at rest. This redistribution of vascular resistance apparently implies that P_c in the post-exercise period is *lower* than in the control period before work and such a drop of P_c can aid in the reabsorption of the fluid that has accumulated in the extravascular space of muscle during exercise (*cf.* Chapter IV).

Since in the different experiments SAP and SVP measurements were obtained from various levels in the vascular tree, the collected data permit a description of the changes in the pressure drop curve from artery to vein induced by exercise. The level at which small pressure was derived in the individual experiment could be defined by the control value for R_P/R_T observed in the muscle vascular bed at rest (" R_P/R_T control"). In Fig. 16 the pressure drop from the popliteal artery to vein is plotted against the calculated values for (R_P/R_T) control. The values for (R_P/R_T) control, which ranges from zero (popliteal artery) to 1.0 (popliteal vein) are plotted on a linear scale on the horizontal axis. The vascular bed is thus for the resting control state divided arbitrarily into "equi-resistant" units implying that for this state the pressure drop curve from popliteal artery (mean AP 105 mm Hg) to popliteal vein (mean VP 5 mm Hg) forms a straight line. For reasons discussed below, mean hydrostatic capillary pressure in the control state was considered to be 22 mm Hg, on the average, and the pressure drop along the capillaries 6 mm Hg. By these values the capillary section can be delimited on the horizontal axis in the diagram of Fig. 16 and, hence, also the precapillary and postcapillary vascular sections. Implicit in the method of seg-

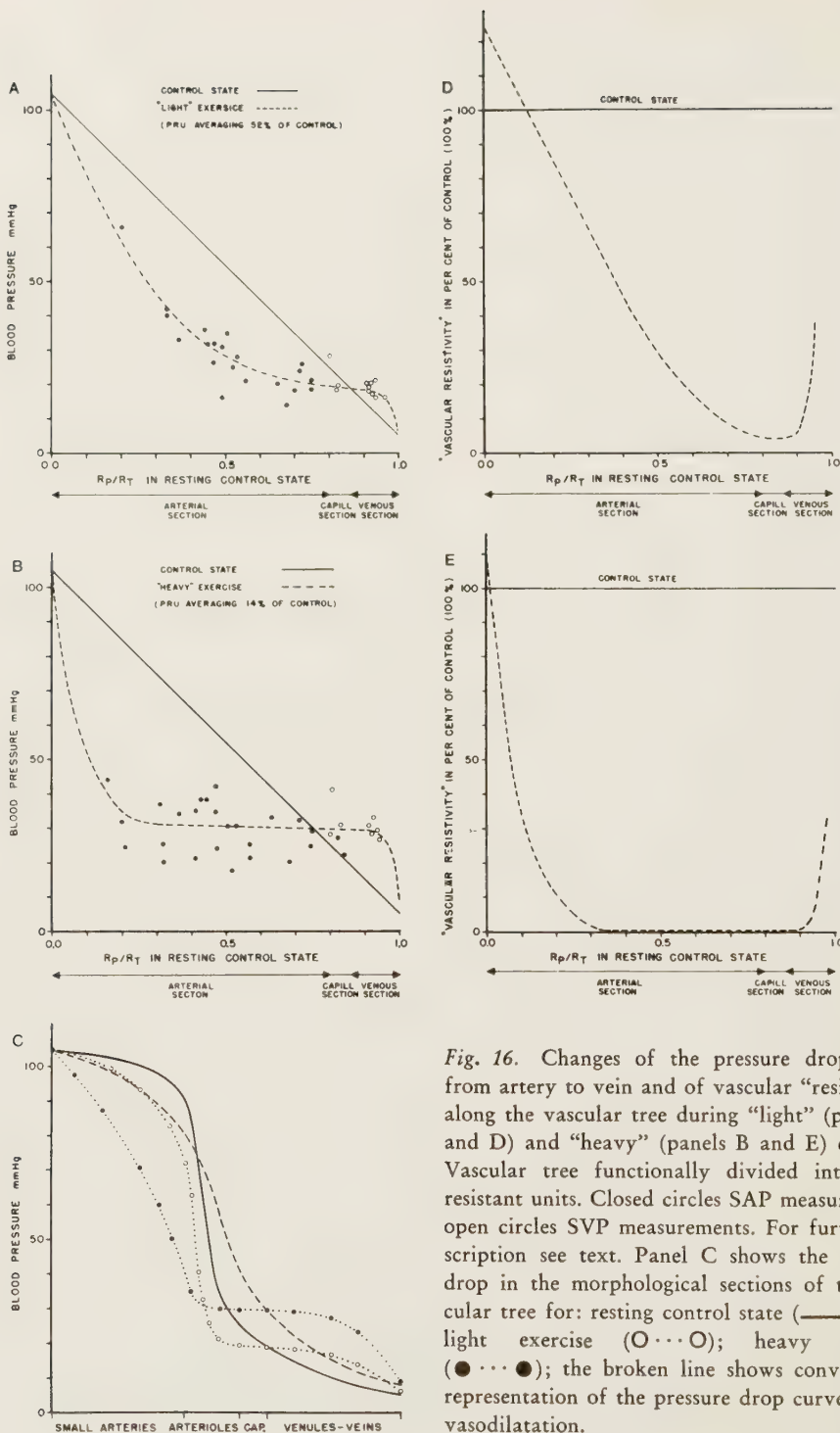


Fig. 16. Changes of the pressure drop curve from artery to vein and of vascular "resistivity" along the vascular tree during "light" (panels A and D) and "heavy" (panels B and E) exercise. Vascular tree functionally divided into equi-resistant units. Closed circles SAP measurements, open circles SVP measurements. For further description see text. Panel C shows the pressure drop in the morphological sections of the vascular tree for: resting control state (—); light exercise (O ··· O); heavy exercise (● ··· ●); the broken line shows conventional representation of the pressure drop curve during vasodilatation.

mental pressure recording is that small vessel pressure during experimental intervention is derived from the same site as in the control situation. Thus, in Fig. 16, the individual observations of small artery and small vein pressures during exercise have been plotted against the given corresponding values for (R_P/R_T) control. As mentioned above, the induced small pressure changes varied with the decline in R_T , reflecting work intensity. Therefore the material has been classed and the plotted data refer to two levels of R_T change, panel A to experiments in which R_T decreased by less than 60 % ("light exercise") and panel B to experiments in which R_T decreased by more than 80 % ("heavy exercise") of resting control resistance; (it may be mentioned that the spread of data in the diagrams can partly be attributed to the variations of the induced change of R_T that still were present in each class of data). In these diagrams the values for VP during exercise refer to the mean value of all observations in each group of experiments. Since AP varied slightly between the individual experiments, all values for SAP and SVP have been corrected in the diagrams so as to be relevant for the mean AP of 105 mm Hg using the experimentally found R_P/R_T values in the recalculation. Test experiments, in which the influence of a mechanically produced change in AP on small vessel pressures was observed directly, showed that the error introduced by such a recalculation was quite insignificant. The smooth curves in the diagrams show the approximate shapes of the pressure drop curve from artery to vein during light and heavy exercise. The curves are fitted by eye over the range of experimental observations and extrapolated further to the AP and VP values. It can be seen that exercise caused a marked shift of the pressure drop curve. Thus, the pressure drop in proximal arterial and distal venous vessels was greatly increased, whereas concomitantly that across the small vessels became reduced. These changes were more pronounced the heavier the exercise. The curves further indicate that mean hydrostatic capillary pressure (P_c) was largely unaltered (or even decreased) during light exercise but that it rose by some 8 mm Hg during heavy work. Note again that the effect on P_c of the elevated SVP to a significant extent was balanced by the concomitant drop of precapillary small vessel pressure. The pressure drop curve for those experiments in which the decline in R_T was greater than in panel A but less than in panel B, "moderate work", is omitted due to limited space in the diagram. As expected, this curve was situated between the curves in panels A and B and with regard to the capillary pressure it indicated that P_c was increased by some 3 — 4 mm Hg.

It should be pointed out that the vascular tree in panels A and B of Fig. 16 with regard to resistance has been considered from a functional point of view and that here it was divided into "equi-resistant" units. These data cannot be directly transformed so as to depict the pressure drop in the various consecutive morphological sections of the muscle vascular bed (arteries, arterioles, precapillary sphincters, capillaries, venules, and veins) since detailed concomitant morphological and functional studies are still lacking. No doubt the major pressure drop in

the resting vascular bed occurs in the small precapillary vessels (especially in the arterioles). The pressure drop curve (solid line) in panel C of Fig. 16, familiar from textbooks etc, may thus reflect the situation for a muscle vascular bed (feeding artery to draining vein) with normal tone. Since this curve corresponds to the straight line control pressure curve in panels A and B (with the reservation that the vascular bed was denervated in the present study) it is possible to rearrange the data observed during exercise (smooth curves in panels A and B) so as to depict in approximate terms the pressure drop across the various morphological vascular sections (panel C, light exercise open circles, heavy exercise closed circles). Thus the major pressure drop during vasodilatation evoked by heavy exercise apparently occurs in small and medium-sized arteries instead of in the arterioles. The broken line shows how the pressure drop curve during vasodilatation, in principle, has been visualized conventionally (*cf.* Guyton 1966, Folkow and Neil 1971). The present report, in which a more systematic study was performed of the pressure drop in various segments along the vascular tree, strongly suggests that this conventional curve may not be correct at some critical points. It should be pointed out in this connection that the pressure drop curve seemed quite similar to that described for exercise when, in an additional series of experiments, vasodilatation was produced in the resting skeletal muscle by close arterial infusion of papaverine or acetylcholine.

The pressure drop curves presented in Fig. 16 enable the calculation, in approximate terms, of the changes in resistance in response to exercise in any delimited part of the vascular bed. Such treatment of the data would give a more refined analysis than the above described changes of gross segmental resistance. Although each curve in panels A and B gives information about the distribution of resistance to flow from artery to vein at the prevailing R_T levels, the evoked changes of resistance by exercise in the various vascular sections cannot be evaluated unless the changes in blood flow are taken into consideration. The blood flow values observed in the control states and during light and heavy exercise were recalculated to be valid for the perfusion pressures ($AP - VP$) in panels A and B; (it should be noted that the error introduced by such a recalculation is small when the variations in AP is limited, *cf.* Chapter II, Results Section A 3). It was found that, from a mean resting control blood flow of $7.8 \text{ ml/min} \times 100 \text{ g}$, flow on average increased by a factor of 2.0 during light exercise. During heavy work it increased by a factor of 6.8 from a mean resting value of $7.1 \text{ ml/min} \times 100 \text{ g}$. It is clear that for each given state, each section of the vascular bed is traversed by the same volume flow (apart from smaller differences due to net transcapillary fluid exchange, see below). Resistance at any point, denoted "vascular resistivity", may therefore be obtained from the slope of the curves relating pressure (P) to (R_P/R_T) control (Fig. 16, A and B) and from the blood flow according to the equation:

$$\text{"resistivity"} = - \frac{dP}{d[(R_P/R_T) \text{ control}]} \cdot \frac{1}{\text{flow}} ;$$

The changes of vascular resistivity induced along the vascular bed during light and heavy exercise are shown in panels D and E of Fig. 16 with the exception of the very distal part of the venous segment which was omitted due to the difficulty in extrapolating the pressure curves (Panels A and B) down to VP. Since, for the control state, the vascular tree was divided into "equi-resistant" units (straight line in Fig. 16 A and B), control resistivity is constant throughout the vascular bed and has been set to 100 per cent. The resistivity during exercise is expressed in per cent of control. Therefore exercise values below 100 per cent denote a decrease of resistivity and values above 100 per cent an increase of resistivity compared to the control state. The diagrams give a graphical description of the changes of resistivity along the entire vascular bed and the integral of the curves (areas under the curves) depicts the vascular resistance distribution.

It can be seen that light as well heavy exercise induced a decrease of vascular resistivity in all but the most proximal parts of the vascular tree. During a relatively small dilator response, as produced by light exercise, the most pronounced changes of resistivity occurred in the microvessels, whereas during heavy exercise marked resistivity changes also occurred in the somewhat larger vessels, especially on the precapillary side. These changes can be ascribed mainly to relaxation of the vascular smooth muscle, the effect in the capillary section being related to an opening up of "precapillary sphincters" greatly increasing the number of parallel-coupled capillary channels. It appears that rheological effects, *e.g.* decreased blood viscosity, can contribute to the resistivity decline (see Comments). In the somewhat larger venous vessels ("capacitance vessels") the decrease of resistivity may be caused mainly by passive distension due to the increased transmural pressure (*cf.* Chapter II, Results section C). The increased resistivity in the most proximal arterial vessels during light exercise is in agreement with the changes of segmental resistance described above (Table III) and the present analysis suggests that such an effect may be present also during heavy exercise in the vessels quite close to the conduit artery. The drastic decrease of resistivity in the small vessels during heavy exercise might at first sight appear surprising but seems plausible in view of the fact that R_T decreased by more than 80 % and that resistivity decreased much less in other sections of the vascular bed.

B. Hypertonic infusion experiments

These experiments were performed on preparations in which total vascular resistance (R_T) in the control period before infusion was of about the same magnitude as in the above described exercise experiments and in which the hypertonic infusion rates were adjusted so as to elicit graded decreases of R_T over approximately the same range as encountered in the exercise experiments. The experimental hyperosmolality was produced by *i.a.* infusion of glucose or sodium lactate solutions which were found to elicit similar effects.

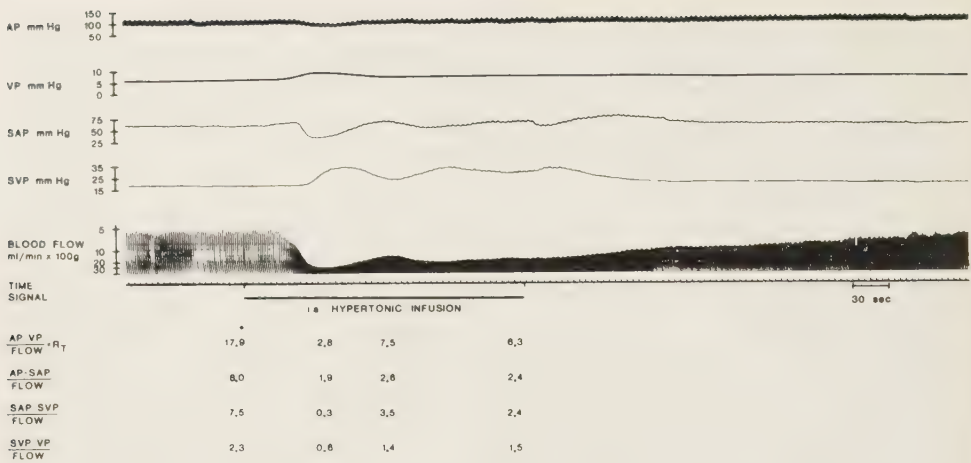


Fig. 17. Tracings of blood flow and of arterial inflow pressure (AP), venous outflow pressure (VP), small artery pressure (SAP), and small vein pressure (SVP) in resting muscle during hypertonic infusion. Calculated values for total resistance (R_T) and for resistances in various segments of the vascular bed shown at bottom of the figure.

Fig. 17 shows representative original tracings of blood flow and of the various vascular pressures during hypertonic glucose infusion (signal). At the bottom of the record, calculated values for R_T and the segmental resistances are given. It can be seen that the infusion elicited a typical pattern of blood flow increase (*cf.* Chapter II, Fig 5), *i.e.* an initial peak response followed by some return of flow (steady state response). Thus, flow increased from a control value of 5.6 ml to 29.3 ml at the peak and to 12 — 15 ml/min $\times$ 100 g tissue in the steady state phase. AP was 106 mm Hg in the control period and remained at about this level throughout the experiment except for a slight transient fall early during infusion. VP increased by a few mm Hg from a control value of 6 mm Hg. SAP was 61 mm Hg in the control period corresponding to an R_P/R_T value of 0.45. During the hypertonic infusion there was an initial slight, transient increase of SAP, possibly an effect of the fluid infusion *per se* and of the evoked transcapillary fluid absorption. SAP then showed a rapid fall and reached a low level of 35 mm Hg by the time the peak flow response was almost but not fully developed. This decrease mainly can be ascribed to a relatively more pronounced dilatation of the vessels located distal to the point of measurement than in the proximal vessels and to a slight extent to the concomitant fall of AP. It should be noted that at the time blood flow was approaching its peak value, SAP started to increase again and rose by about 5 mm Hg during the short phase when blood flow was maintained constant at this high level. This effect which is more clearly shown in the next figure, can be ascribed to a progressive resistance decrease in the proximal vessels soon followed by the commencement of a resistance increase in the distal vessels. SAP then showed a more pronounced increase concomitant

to the gradual return of flow towards the steady state level and reached a value of 69 mm Hg in the early steady state phase after which it stabilized at about the control value. This shows that the return of flow from peak to steady state is due to a relatively more pronounced partial recovery of resistance in the distal than in the proximal segment which is also clear from the figures for absolute resistances. SVP increased from a control value of 19 mm Hg to a maximal value of 34 mm Hg and it stabilized after some oscillation at a value of about 30 mm Hg during the infusion. The SVP increase was related to a relatively more pronounced resistance decline in the vessels proximal than distal to this site of pressure measurement. Upon cessation of the infusion blood flow and the pressures gradually returned to the control values. Thus the redistribution of vascular resistance observed after exercise was not present. The small initial changes of SAP and SVP at the end of the infusion may be related to osmotic transcapillary fluid flux into the tissue occurring when the infusion ended. Summarizing, Fig. 17 shows that the decreases of R_T in the peak and the steady state phases can be ascribed to dilator effects in all the three vascular segments. During the peak response the relative change in resistance is most pronounced in the vessels located distal to the SAP measurement although the proximal segment contributes to an almost equal resistance decline in absolute terms. Recovery of vascular tone from peak to steady state response is mainly due to constriction of the small vessels.

Fig. 18 shows changes of blood flow and SAP during hypertonic infusion in an experiment where the SAP measurement was derived from a more distal point ((R_P/R_T) control = 0.72). This tracing may serve to illustrate the not infrequently observed finding that after a transient fall of SAP during the development of the flow increase (in this case from 40 to 27 mm Hg), there was a marked increase of SAP at a time clearly preceding that at which blood flow started to return to the steady state level (in this case from 27 to 48 mm Hg). When the steady state flow was established, SAP significantly exceeded its control value. This was the usual finding in experiments in which SAP was obtained from the smallest precapillary vessels. The extent to which R_T , R_D , and R_P changed during various phases of the hypertonic infusion is shown by the figures at bottom of the record. Since AP and VP were not much changed, the alterations of SAP can mainly be ascribed to the changes of R_D/R_P . The values for R_D and R_P and for R_D/R_P shows that during the early dilator response, vascular resistance declines in both the proximal and distal segments, and that the relative change is greatest in the distal one. During the maximal flow increase there is a continuing resistance decline in the proximal segment whereas distal resistance starts to increase again, both these effects contributing to the increased R_D/R_P value. Return of flow to the steady state mainly depends on increased distal resistance. Thus the rise of transmural pressure in the small vessels, which is probably reinforced by a concomitant decrease of tissue pressure due to the osmotic transcapillary fluid absorption, is a phenomenon which starts to develop early during

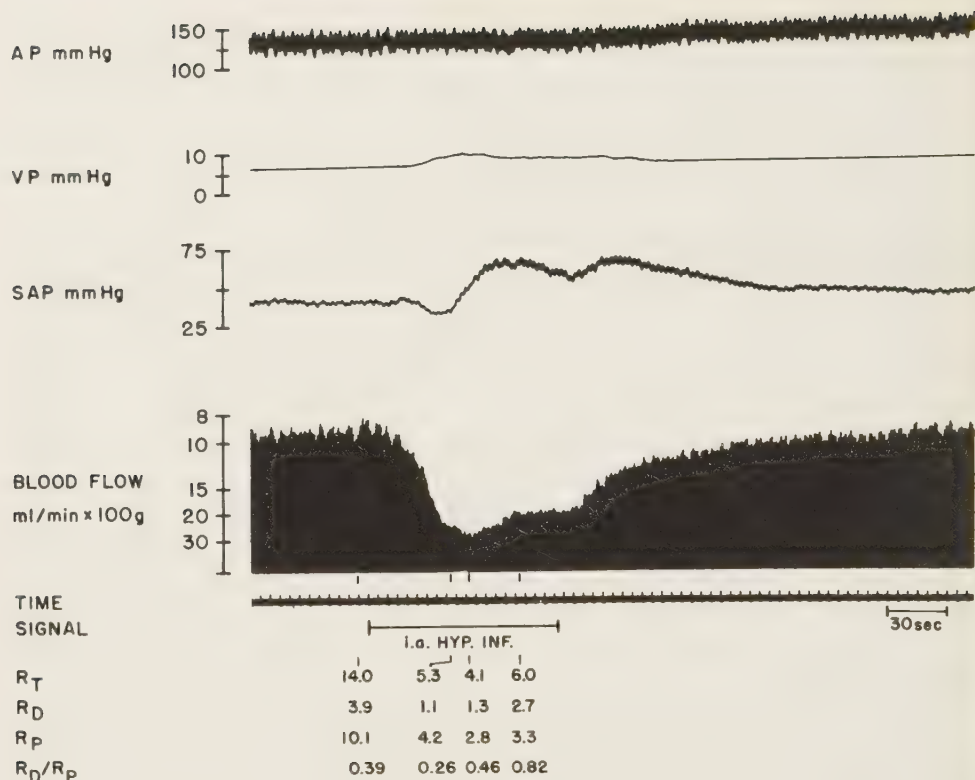
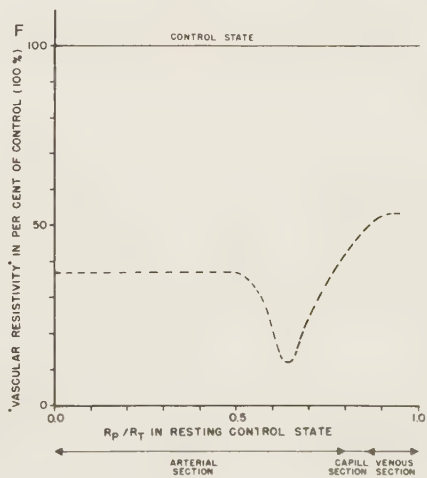
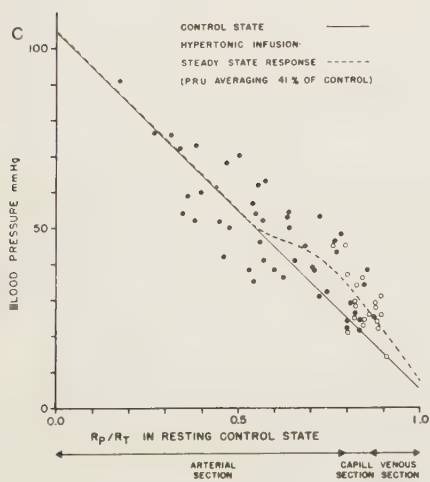
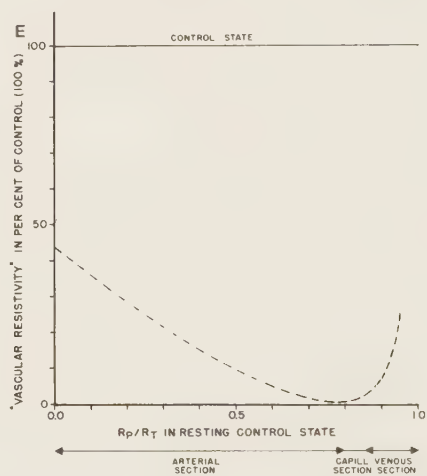
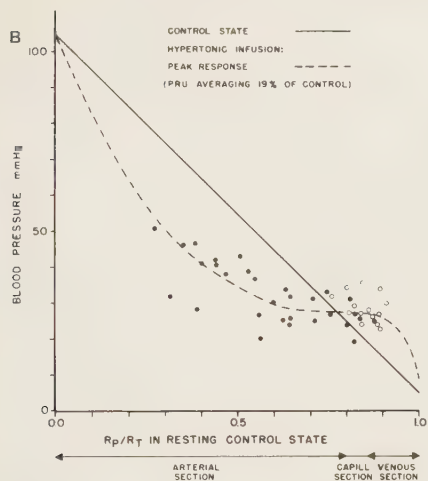
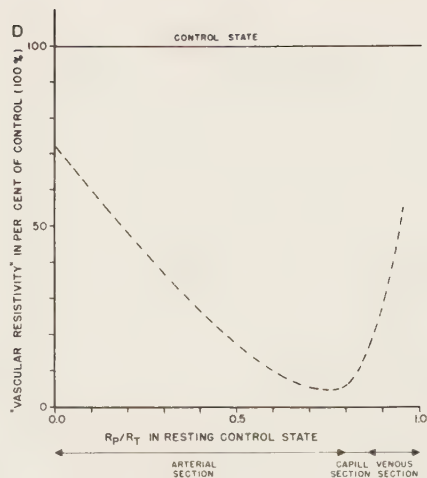
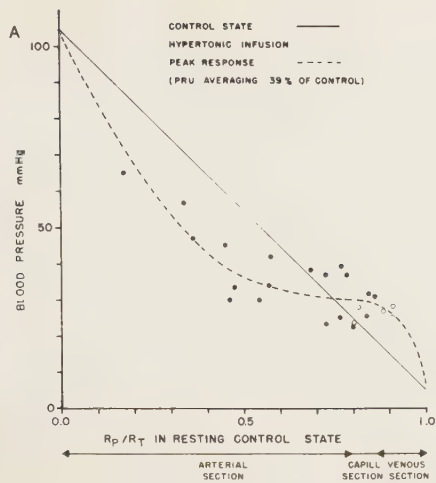


Fig. 18. Tracings of blood flow and of arterial inflow pressure (AP), venous outflow pressure (VP), and small artery pressure (SAP) in resting muscle during hypertonic infusion. Calculated values for total resistance (R_T), for resistance proximal (R_P) and distal (R_D) to the point in the vascular tree of the SAP measurement, and for the ratio R_D/R_P shown at bottom of the figure. Note the marked SAP increase commencing during the peak flow response to hypertonic infusion which tentatively can explain the later partial recovery of vascular tone (steady state flow response) by the elicitation of a secondary myogenic constrictor effect.

Fig. 19. Changes of the pressure drop curve from artery to vein and of vascular "resistivity" along the vascular tree during hypertonic infusion. Panels A and D: "peak response", data for "moderate" decrease of R_T . Panels B and E: "peak response", data for "large" decrease of R_T . Panels C and F: "steady state response". Vascular tree functionally divided into equi-resistant units. Closed circles SAP measurements, open circles SVP measurements. For further description see text.

hypertonic infusion. As will be discussed below, this transmural pressure rise might elicit a myogenic constrictor response in these vessels counteracting the dilator action of hyperosmolality and, hence, at least partly be responsible for the return of blood flow from the peak to the steady state level and, further, for the maintenance of flow (vascular tone) in the later phase. It should be stressed that such a transmural pressure increase was not observed during exercise.



As during the exercise experiments described above small vessel pressure measurements (SAP as well as SVP) were obtained from various points along the vascular bed from artery to vein as shown by the different values for (R_P/R_T) control. The collected data during the hypertonic infusion experiments are shown in Fig. 19 and are presented in a similar way to that used for exercise (Fig. 16), *i.e.* the pressure drop along the vascular bed has been plotted against (R_P/R_T) control (panels A, B and C). Curves for evoked changes of "vascular resistivity" during hypertonic infusion have also been derived (panels D, E and F). As in the exercise diagrams (Fig. 16), resistivity changes in the most distal venous vessels have not been calculated. Panels A and B represent the pressure data during peak response, panel A for experiments in which R_T decreased by less than 75 % (mean decrease 61 %) and panel B for experiments in which R_T decreased by more than 75 % (mean decrease 81 %). These changes of R_T are roughly comparable to those evoked in the exercise experiments shown in panel A and B of Fig. 16. Panel C shows the corresponding data during the early steady state response, *i.e.* in a phase about 1 to 3 min after the start of the hypertonic infusion. Since in this phase of the dilator response to hypertonic infusion no significant systematic difference with regard to small vessel pressures was found when the R_T decline was large or when it was small, the plotted data refer to all the observations (mean decrease of R_T being 59 %).

It can be seen from Fig. 19 that during the peak response (panel A and B) the general characteristics of the pressure drop curves along the vascular bed were similar to those observed during light and heavy exercise (*cf.* Fig. 16). However, during the more pronounced decrease of R_T (Panel B) the pressure drop in the proximal arterial vessels was smaller and in the more distal arterial vessels greater than during heavy exercise. Further, it can be seen that pressure in the smallest precapillary vessels tended to be somewhat higher during the infusion experiments than during exercise, especially at the smaller R_T decline. The curves for "resistivity" changes during the peak response roughly resemble those derived during exercise, although some differences seem apparent. Thus, hypertonicity seems to affect the more proximal arterial vessels to a greater extent and the more distal arterial vessels to a somewhat lesser extent than exercise. With regard to the steady state response during hypertonic infusion, the pressure drop curve from artery to vein largely followed the control curve, and was thus clearly different from those depicted for the peak response and for exercise. It can be inferred from the shape of this pressure drop curve that, although R_T on the average was significantly less than in the control state, the distribution of resistance along the vascular bed during the steady state response was not much different from that in the control state. Consequently, the "resistivity" curve for the steady response showed a virtually constant change of resistivity throughout the vascular bed with the exception for the smallest vessels where it decreased more than in the larger vessels. The difference in the shape of the resistivity curves between the peak and the steady state response could be ascribed mainly to the

preferential constriction of the smaller vessels (apparently including the "pre-capillary sphincters") that occurs during the latter response (*cf.* Figs. 17 and 18). This was also evident when calculating separately the resistivity changes for the steady state response observations in the infusion experiments from which the peak response data in panels A and B, respectively, were derived.

COMMENTS

In the present study the changes of pressure and of resistance along the skeletal muscle vascular bed in response to exercise and hypertonic infusion were defined in greater detail than in previous literature (*cf.* Haddy *et al.* 1954, Folkow *et al.* 1971, Gazitua *et al.* 1971). The experimental approach used is based in principle on the well-known method of Haddy *et al.* (1954) for small vessel pressure measurements and segmental resistance calculations. The processing of data in the present study seems, however, to provide much more detailed information. The validity of the resistance calculations based on measurement of small vessel pressures has been discussed at length by others (*e.g.* Folkow *et al.* 1971). Only a few comments will therefore be made.

In most studies of segmental vascular resistance constant flow perfusion has been used which has the advantage that resistance effects are reflected directly by the observed pressure changes. An obvious drawback with constant flow perfusion is, however, that unphysiologic pressure changes are induced; during vasodilatation, for instance, AP often falls to such low levels that secondary passive alterations in vessel diameter to an abnormal extent might interfere with the active vascular response. The more physiological approach with constant pressure perfusion was used in the present study.

The same volume flow of blood should pass each consecutive section of the vascular bed at a given moment and it is assumed with this method that there is a uniform distribution of flow in the various sections. This assumption seems reasonable for the larger pre- and postcapillary vessels but, as pointed out by Folkow *et al.* (1971), blood flow in the smaller vessels may show considerable random variation. Therefore, results from a single experiment may not necessarily be conclusive but, as stressed by these authors, uniform results from a large series of experiments, as in the present study, should provide relevant information; (Mention should be made here that as a result of the net transcapillary fluid fluxes which are evoked by exercise and by hypertonic infusion, the volume flow in pre- and postcapillary vessels was in all probability somewhat different in the present study (*cf.* Chapter II). The consequences of this for the results presented will be considered below). Another assumption is that small vessel pressure is transmitted through the same collateral vessel in the control situation and during experimental intervention; this appears likely but cannot be proved. Again, collecting data from many experiments would seem to minimize the error, if the above should not always be true. Another question of importance, especially when constant pressure perfusion is used, is if kinetic energy forms a significant and

variable part of the total energy, which could affect the pressure measurements and give rise to erroneous conclusions. Calculations of this factor for the various flow rates encountered indicated that such an error must be insignificant, which is further supported by the critical analysis of the problem presented for instance by Burton (1966).

Attempts were made to obtain small vessel pressures from widely different points in the vascular tree by cannulation of various vessels. The large range of small vessel pressures in the control state indicates that the pressure were monitored from arterial and venous vessels of quite varying bores although not from the largest ones, perhaps since these have few collaterals. To some extent the variations of small pressure in the control state would seem caused by resistance variations. Such an influence was minimized, however, by accepting only those experiments in which control R_T could be considered "normal".

SAP values, say below 35 mm Hg, may be considered representative for quite small precapillary vessels, and the control observations below this pressure level averaged 26 mm Hg (11 animals; mean AP 104 mm Hg and mean VP 6 mm Hg). SVP averaged 20 mm Hg (9 animals; mean AP 109 mm Hg and mean VP 6 mm Hg). Control mean capillary pressure ought to be somewhere in between these values and probably closest to the SVP; the figure of 22 mm Hg for P_c used in the diagrams (Figs. 16 and 19 above) thus seems reasonable and is in rough agreement with data given in the literature (e.g. Landis and Pappenheimer 1963, Smaje *et al.* 1970) although there are reports suggesting that P_c may be as low as about 15 mm Hg (e.g. Diana 1970). The figure of 6 mm Hg used for the pressure drop across the capillary is in accordance with findings on rat cremaster muscle (Smaje *et al.* 1970). It should be pointed out here that due to possible variation of blood flow in the small vessels, observations of an especially low SAP or an especially high SVP do not necessarily provide information about mean P_c for *all* capillaries in the preparation.

The collected data on blood pressures and flow provided rough information about changes of the pressure drop curve and of resistance alterations in delimited parts of the vascular tree induced by exercise or hypertonic infusion (Figs. 16 and 19). It is evident, as can be expected, that the major resistance decline occurred in the small vessels. The surprisingly large decline even in the capillaries deserve comment; (it may be noted that should resting control P_c have been overestimated, say by some 5 mm Hg, the resistivity decline in the capillary section during heavy exercise would still be pronounced as suggested by Fig. 16). In the denervated vascular bed of the cat lower leg muscles the capillary filtration coefficient (CFC), reflecting the number of patent capillaries, increases by some 200 — 300 per cent during heavy exercise (e.g. Kjellmer 1964 *a*, Lundvall *et al.* 1967, Chapter II and IV this volume). Assuming the mean diameter and mean length of the capillaries opened up during exercise to be similar to those in the control state, these changes in CFC would correspond to a resistance decrease in the capillary section down to about $1/4$ of the control values. Some other fac-

tors might contribute to the observed resistance decline, for instance a possible viscosity decrease as flow velocity increases. Alternative explanations could be a possible distension of the capillaries, opening of arterio-venous shunt vessels etc, but there is experimental evidence that this does not occur in skeletal muscles (for ref. see Mellander and Johansson 1968).

Somewhat puzzling is the quite regular observations that, especially during light exercise, there seemed to be some resistance increase in the most proximally situated arterial vessels (e.g. Table III). There is at present no explanation for this phenomenon even if it is likely that it is caused by passive factors. For instance, "nipping" of these vessels caused by muscle contraction (*cf.* Gray *et al.* 1967) or a passive elastic recoil due to the fall of transmural pressure could possibly have contributed to the effect.

A reason for undertaking this series of experiment was to get some indication of which of the resistance responses during hypertonic infusion, the peak or the steady state, should be considered in the quantitative evaluation of the role of hyperosmolality in exercise hyperemia. The one that showed a pattern of resistance response within the various parts of the vascular tree best resembling that evoked by exercise would seem of greatest relevance, especially if a deviating pattern could be regarded as caused by an "experimental artefact" (see below). It is clear from the present results (e.g. Figs. 16 and 19) that the peak effect was roughly similar to that during exercise, whereas the steady state effect differed markedly. It should be pointed out that as a result of transcapillary fluid transfer into the muscle during exercise and into the blood stream during hypertonic infusion, precapillary blood flow must have been underestimated during work but overestimated in the infusion experiments (Chapter II, A 1 and A 2). Since this error should have been of greatest significance during the peak response to hypertonic infusion the main consequence would be that the resistivity changes during exercise and during the peak response in fact are more alike than indicated by the presented data. The deviating resistance pattern during the steady state response was caused by a partial but clear-cut recovery of vascular tone, preferentially in smaller vessels, upon transition from the initial peak response. A tentative explanation for this phenomenon is that a myogenic constrictor effect develops in response to raised transmural pressure in the small vessels. An increase of intraluminal pressure could thus be observed shortly before the partial recovery of tone and was marked and maintained during the steady state hyperemic phase (e.g. Fig. 18). The osmotic fluid absorption of extravascular fluid during hypertonic infusion would, in addition, tend to decrease interstitial pressure thereby further raising transmural pressure, and such a decrease of interstitial pressure can apparently be quite significant in response to even small fluid shifts (Guyton 1965). In fact, the addition of fluid at the capillary level may also partly be responsible for the upstream intraluminal pressure increase. A stimulus for a myogenic constrictor response thus seems present during hypertonic infusion. That the small precapillary vessels, at least the "sphincters",

constrict upon increased transmural pressure during hypertonic infusion is shown in Chapter V. To the extent that a high transmural pressure and a consequent myogenic constriction during hypertonic infusion is caused by extravascular fluid absorption *per se* and by a secondary decrease of tissue pressure it can be considered an "experimental artefact" inherent in the infusion experiments, not present when hyperosmolality primarily develops in the extravascular space as during exercise. Since a myogenic response during hypertonic infusion will be elicited with some delay after the stimulus develops it should not be prominent during the peak response. Therefore, this dilator phase during hypertonic infusion may be more relevant than the steady state response when evaluating the role of hyperosmolality in exercise hyperemia (*cf.* Fig. 14, Chapter II).

CHAPTER IV

MECHANISMS INVOLVED IN TRANSCAPILLARY FLUID MOVEMENTS DURING AND AFTER EXERCISE

It is well-known that during exercise there is a transcapillary fluid movement from the intravascular space into the extravascular compartment of the active muscles. Under certain circumstances, this process will be of great importance for general cardiovascular dynamics. Thus it has recently been shown that heavy exercise with large muscle groups can lead to a fluid accumulation in the active muscles of more than 1.000 ml in the human being and further that compensatory mechanisms are put into play so as to keep the consequent plasma volume reduction within tolerable limits (Lundvall *et al.* 1970, 1972). The mechanism behind the transcapillary fluid flux into exercising muscles was studied by Kjellmer (1964a) who arrived at the conclusion that it was solely due to filtration caused by increased capillary hydrostatic pressure. Our finding that exercise leads to considerable muscle tissue hyperosmolality (Mellander *et al.* 1967, Chapter II this volume) strongly indicated that an osmotic process was also involved. The aim of the present study was to investigate the relative importance of these two mechanisms for the fluid transfer into exercising muscle. An attempt was also made to reveal the factors responsible for fluid return into the circulatory system after the cessation of work.

METHODS

41 cats (2.7—5.1 kg) of both sexes, anaesthetized as described in Chapter II, were used in this study.

The experiments were performed on the lower leg muscles which were enclosed in a water-filled plethysmograph (38°C) permitting continuous recording of tissue volume. For surgical and technical details see Chapter II. Arterial inflow pressure and regional venous outflow of blood were recorded continuously as described (Chapter II). Most of the experiments were performed under constant pressure perfusion. In some of these, the arterial inflow was diverted from the ipsilateral femoral to the popliteal artery through a shunt circuit to permit intra-arterial infusions proximal to an inserted "mixing chamber" (Chapter II). In some experiments blood flow was controlled by the use of a perfusion pump (Harvard) on the arterial side.

Muscle exercise was produced by electrical stimulation (5V, 0.5 msec, 0.25—4 imp/sec) of the distal end of the severed sciatic nerve using a Grass stimulator. Observations were made during graded "short-term" (<5 min) and "prolonged" ($\approx$ 15 min) exercise.

Some observations of the transcapillary fluid flux in resting muscle induced by hypertonic (≈ 1500 mOsm/kg) glucose infusion (0.1–1.0 ml/min) were also made. Infusion of papaverine hydrochloride (>0.6 mg/min) was sometimes given to produce a maximal dilatation of the vascular bed.

Blood samples (<1.5 ml) were taken for plasma osmolality determinations from a T-tube in the venous outflow tubing close to the popliteal vein and from a catheter in the right subclavian artery (exercise experiments) or from a T-tube in the arterial shunt distal to the mixing chamber (hypertonic infusion experiments) after discarding the "dead space" fluid volumes. To permit determination of the arterio-venous osmolar difference across the muscle vascular bed, arterial and venous blood samples were withdrawn simultaneously in the exercise experiments whereas in the hypertonic infusion experiments the sampling had to be separated slightly in time, the arterial sample being taken immediately after the venous sample. The osmolality measurements and compensation for the sampled blood volumes were performed as described in Chapter II.

Tissue volume was continuously recorded on a kymograph by the use of an air-filled piston recorder connected to the plethysmograph. For the present purpose this mechanical recorder system showed greater sensitivity and stability than currently available electronic units. The sensitivity of the volume recording system could be varied so that a volume shift of 0.1 ml (corresponding, on the average, to about 0.15 per cent of the studied tissue volume) gave a deflection in the recording of 3–9 mm. By adjusting the height of the orifice of the venous outflow tubing from the muscle region, venous outflow pressure could be controlled. In the beginning of the experiments, venous outflow pressure was adjusted so that tissue volume was constant (isovolumetric state). Venous pressure was maintained at this level throughout the experiments except when otherwise stated or when the capillary filtration coefficient (CFC) was determined (see below).

Changes of tissue volume can depend on alterations in the regional blood content (capacitance responses) and/or on alterations in the extravascular fluid volume, the latter caused by net transcapillary fluid movement or lymph drainage. In the present study lymph drainage was prevented by ligation and cauterization of the regional lymph vessels. A large number of previous studies with the present technique have shown that under these circumstances the effects on tissue volume caused by transcapillary fluid fluxes and by changes of regional blood volume can be separated (for ref. see Mellander and Johansson 1968). In most cases this is quite easy since the time courses of the two phenomena are very different. Thus capacitance responses usually develop rapidly and are coordinated in time with the responses of the resistance vessels. It follows that net transcapillary fluid movements can be adequately determined as soon as a steady state vascular response is reached.

Data on the transcapillary hydrodynamic conductivity and changes in functional capillary surface area in the studied vascular bed were derived from de-

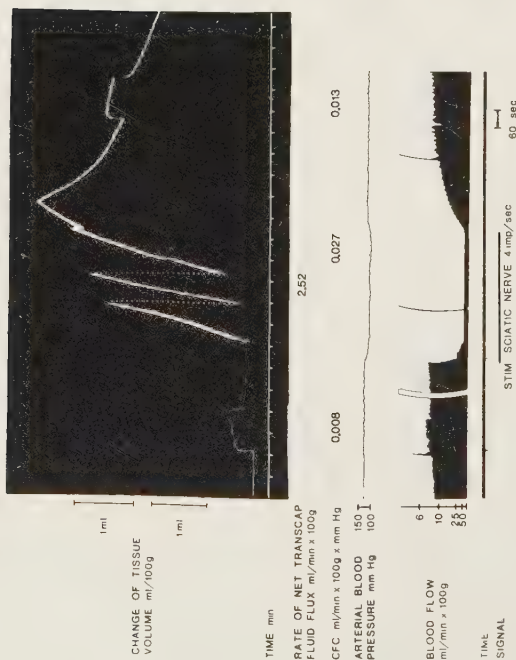
termination of the CFC, performed as described in Chapter II. For the CFC calculations, it was assumed that 80 % of the venous pressure rise was transmitted to the capillary level during constant pressure perfusion (*cf.* Cobbold *et al.* 1963) and 100 % when flow was controlled by the perfusion pump (*cf.* Kjellmer 1964 *a*). Muscle tissue weight was determined after dissection (see Chapter II).

RESULTS

The experiments illustrated in Fig. 20 were designed so as to permit an approximate quantitative evaluation of the net transcapillary fluid flux during exercise on the one hand caused by increased hydrostatic capillary pressure (filtration) and of the other caused by osmosis. Panel A shows representative changes of tissue volume and of blood flow in the lower leg muscles during relatively short-term heavy exercise (4 imp/sec). The capillary filtration coefficient (CFC) and arterial and venous osmolality was determined before and during the work. Blood flow was 7.2 ml/min $\times$ 100 g tissue in the resting control period and it increased rapidly at the commencement of exercise reaching a maximal and steady state level of 50.0 ml/min $\times$ 100 g after about 50 sec. Tissue volume, which was approximately constant in the control period (isovolumetric state), showed a most pronounced increase during exercise (volume recorder lowered twice during the period of work). A small fraction of the volume increase can be ascribed to an augmentation of regional blood volume (capacitance response, mainly passive, *cf.* Chapter II), an effect almost fully established at the time blood flow reached its maximal level (see Methods). The large tissue volume increase occurring during the phase of the steady hyperemia is therefore almost entirely due to a net transcapillary fluid movement from the intravascular to the extravascular compartment and it amounted in total to about 7.90 ml/100 g tissue in the 7 min work period (correction made for the effect of the CFC determination). The rate of transcapillary fluid flux was most rapid some two to three min after the commencement of exercise and then amounted to 2.52 ml/min $\times$ 100 g after which it tended to decline somewhat. It can be seen that exercise led to an increase of CFC from a control value of 0.008 to 0.027 ml/min $\times$ 100 g $\times$ mm Hg. At the time of maximal rate of transcapillary fluid movement, venous osmolality had increased by 26 mOsm/kg H₂O, indicating that a considerable osmolar gradient from tissue to blood was caused by the work since concomitantly arterial osmolality increased by only 1 mOsm/kg H₂O. Upon cessation of the exercise, blood flow, tissue volume and CFC decreased. Blood flow returned to the control level within about 9 min but a large fraction of the accumulated fluid was still present in the muscles.

The extent to which an increase of capillary hydrostatic pressure, by filtration, contributed to the transcapillary fluid flux in the described exercise experiment was approximately assessed from the experiment shown in panel B performed on the same animal. The figure shows the tissue volume changes evoked

A



B

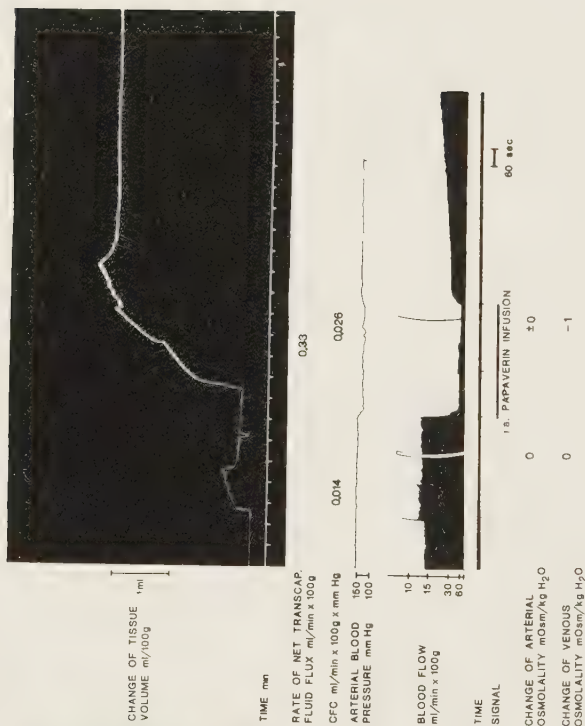


Fig. 20. Net transcapillary fluid transfer into skeletal muscle induced by exercise (panel A) and by i.a. papaverine infusion (panel B), both causing maximal resistance vessel dilatation. Fluid flux represented by the tissue volume increase (after correction for initial capacitance response). The pronounced fluid transfer during exercise can partly be ascribed to fluid filtration, the extent of which is revealed by the infusion experiment; it can mainly be attributed to osmosis caused by work-induced tissue hyperosmolality.

by close i.a. infusion of papaverine administered in a dose so as to elicit a blood flow increase of similar magnitude as in the exercise experiment, *i.e.* a virtually maximal resistance vessel dilatation. It can be seen that papaverine caused quite a marked initial volume increase (capacitance response) concomitant to the development of the hyperemia after which tissue volume increased at a much slower rate (net transcapillary fluid movement). The large capacitance response to papaverine (apparently greater than that evoked by exercise, panel A) indicates that, besides a passive distension of the capacitance vessels, this drug, like acetylcholine, also evokes active dilatation of these vessels (*cf.* Chapter II). The transcapillary fluid movement amounted in total to about 1.50 ml/100 g tissue during the 6 min infusion period (correction made for the effect of the CFC determination), and the maximal rate of fluid flux was $0.33 \text{ ml/min} \times 100 \text{ g tissue}$. This transcapillary fluid flux must be ascribed to filtration caused by an increase of capillary hydrostatic pressure as a consequence of a decrease of the pre-/postcapillary resistance ratio, since, as shown by the figures for arterial and venous osmolality, papaverine apparently caused no transcapillary osmolar gradient. The rate of net fluid filtration is determined by the magnitude of the capillary hydrostatic pressure rise and by the prevailing capillary hydrodynamic conductivity, the latter reflected by the CFC, which was found to be $0.026 \text{ ml/min} \times 100 \text{ g} \times \text{mm Hg}$ during the papaverine infusion.

As described in Chapter III, exercise and papaverine seem to evoke roughly similar changes of capillary hydrostatic pressure (and hence of the pre-/ postcapillary resistance ratio) in the muscle vascular bed at comparable decreases of total regional vascular resistance, despite the seemingly greater influence of papaverine on the capacitance vessels. Apparently the contribution to postcapillary resistance of the main capacitance vessels is small. The rise of capillary hydrostatic pressure in the present exercise and papaverine experiments should therefore be approximately equal since also the changes of the arterial blood pressure were similar in the two experiments. It follows that the net fluid filtration in the two experiments must have been about equally large, since CFC was of the same order of magnitude. Therefore, during the phase of maximal transcapillary fluid movement caused by exercise ($2.52 \text{ ml/min} \times 100 \text{ g}$), less than 15 per cent of the fluid flux can be ascribed to fluid filtration ($0.33 \text{ ml/min} \times 100 \text{ g}$). The remaining larger part of the fluid transfer is obviously due to an osmotic process caused by the transcapillary osmotic gradient developed in the exercising muscles as a consequence of the regional tissue hyperosmolality.

The rise of mean capillary hydrostatic pressure occurring in the papaverine experiment can be calculated approximately by dividing the rate of fluid flux ($0.33 \text{ ml/min} \times 100 \text{ g}$) by CFC ($0.026 \text{ ml/min} \times 100 \text{ g} \times \text{mm Hg}$) which gives a figure of 13 mm Hg; from the foregoing discussion it follows that mean hydrostatic capillary pressure should have risen by the same amount in the exercise experiment. A similar deduction for the exercise experiment (maximal fluid movement divided by CFC) gives a figure of 93 mm Hg ($2.52/0.027$). The "ef-

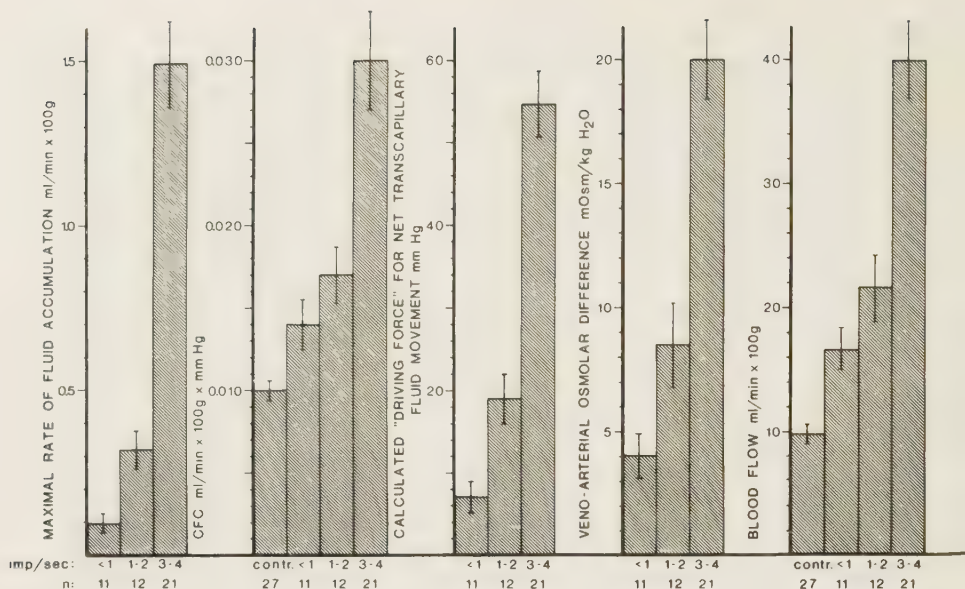


Fig. 21. Diagram showing mean values $\pm$ SEM of the concomitantly determined maximal rate of net transcapillary fluid flux into exercising muscle, transcapillary filtration coefficient (CFC), transcapillary "driving" force for net fluid transfer (fluid flux/CFC), veno-arterial osmolar difference, and blood flow. Data classed with regard to rate of somato-motor fibre stimulation (work "intensity"). n denotes number of observations in each class. CFC and blood flow in the resting control state also shown.

fective transcapillary osmotic force" induced by muscle work thus amounted to about 80 (93—13) mm Hg.

At all frequencies of somato-motor fibre stimulation, reflecting work intensity, the maximal rate of net transcapillary fluid movement from blood to exercising muscles was developed in the time period 2 to 4 min after the commencement of exercise. As will be shown, this can mainly be explained by the fact that the veno-arterial (transcapillary) osmolar difference and the hyperemia had then reached maximal levels. The hydrostatic capillary pressure rise should also have attained its maximum at this time.

Fig. 21 shows mean data $\pm$ SEM from all exercise experiments (n=44) performed under constant pressure perfusion for the following parameters: maximal rate of fluid movement, CFC, calculated driving force for net transcapillary fluid movement, veno-arterial osmolar difference and blood flow. The data refer to the time of maximal transcapillary fluid flux and are classed with regard to the somato-motor fibre stimulation frequency, where rates <1 imp/sec may be considered as "light" exercise, 1—2 imp/sec as "moderate", and 3—4 imp/sec as "heavy" exercise. It can be seen that all the depicted parameters were

related to the stimulation frequency. For the three stimulation ranges, the average rates of fluid movement were 0.10, 0.32 and 1.49 ml/min $\times$ 100 g tissue, respectively, and the corresponding values for transcapillary driving force 7, 19, and 50 mm Hg. The clear-cut veno-arterial osmolar differences suggest that an osmotic flux may be present at each work intensity. The data reported in Chapter III indicated that capillary hydrostatic pressure on the average was largely unchanged during light exercise and rose by some 4 mm Hg during moderate, and by some 8 mm Hg during heavy work. The latter figure is in accordance with a calculated average increase of capillary hydrostatic pressure of 9 mm Hg observed during maximal vasodilatation induced in the present study by i.a. infusion of papaverine ($n=6$). The values for total driving force minus the mentioned figures for capillary hydrostatic pressure rise during exercise gives transcapillary osmotic forces for fluid flux of 7, 15, and 42 mm Hg. More than 75 per cent of the fluid movement thus seems caused by osmosis at any one of the work intensities. Further comments on the data in Fig. 21 will be made later on.

The magnitude of the osmotic fluid movement from blood to the exercising muscles appears to depend on several factors. The fluid flux should be directly related to the established transcapillary osmolar gradient provided substances with similar osmotic reflection coefficients with regard to the capillary barrier are responsible for the interstitial hyperosmolality at different work intensities; (for discussion of reflection coefficient, see Landis and Pappenheimer 1963). The transcapillary osmolar gradient and thus the fluid movement may, in turn, be related to the magnitude of the blood flow through an influence of the latter on the transcapillary osmolar extraction. An attempt to approach these problems was made by investigating the relationships between the fluid flux and the veno-arterial osmolar difference and blood flow respectively, and by a theoretical analysis of the result given in the Comments section. The observations were made in early phases of exercise to minimize interference with the fluid movement from a later gradual tissue pressure increase.

Fig. 22 is a representative original record from a series of experiments ($n = 4$) indicating that the net transcapillary fluid movement during exercise is directly related to the transcapillary osmotic gradient. In these experiments, performed under constant flow perfusion, vascular tone was completely abolished by a continuous i.a. infusion of a supramaximal dose of papaverine. Thus superimposed exercise could not elicit any further changes of vascular tone and, hence, no further increase of hydrostatic capillary pressure. Net fluid *filtration* induced by exercise *per se* was thus prevented. Since blood flow and CFC were constant throughout the experiment (Fig. 22), hemodynamic variables of possible importance for osmotic fluid exchange, such as blood flow, capillary flow distribution, capillary flow velocity, capillary surface area and capillary permeability could be considered constant (see Comments). Under these experimental conditions, the degree of extravascular hyperosmolality was varied by performing

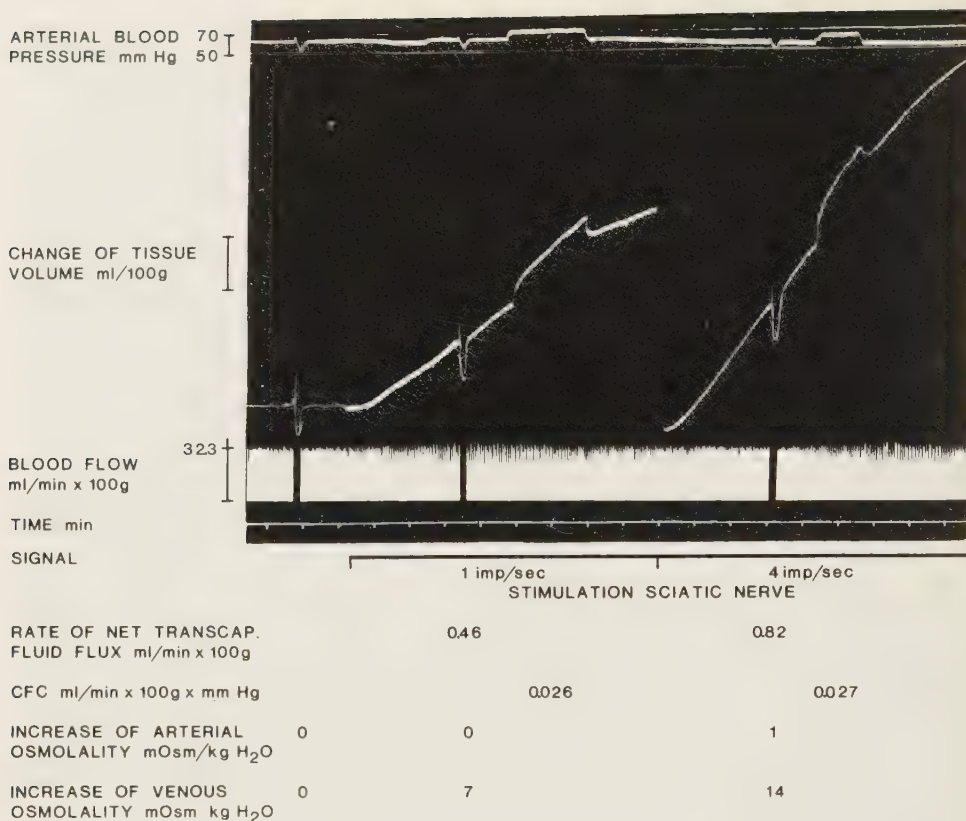


Fig. 22. Experiment indicating that osmotic fluid flux to exercising muscle is proportional to the induced veno-arterial osmolar difference which was varied by performing work at two different intensities (somato-motor fibre stimulation 1 and 4 imp/sec). Vascular tone during the experiment abolished by continuous i. a. infusion of papaverine so that exercise *per se* could not elicit any change of capillary hydrostatic pressure, capillary surface area (CFC) or fluid filtration.

work at two different intensities (somato-motor fibre stimulation at 1 and 4 imp/sec). The veno-arterial osmolar difference induced by work was determined at the two levels of exercise. Under the prevailing experimental conditions, this difference may be directly related to the evoked transcapillary osmolar gradient (see Comments). It can be seen from the figure that stimulation at 1 imp/sec caused an osmotic transcapillary fluid flux which, after about 1 minute, occurred at an approximately steady rate of 0.46 ml/min x 100 g tissue. The induced veno-arterial osmolar difference, determined after about 3 minutes, was 7 mOsm. When the stimulation rate was increased to 4 imp/sec, the maximal fluid flux occurred at a rate of 0.82 ml/min x 100 g and concomitantly the induced veno-arterial osmolar difference was increased to 13 mOsm. The rate of net transcapillary fluid movement was about 1.8 times greater at the higher work intensity,

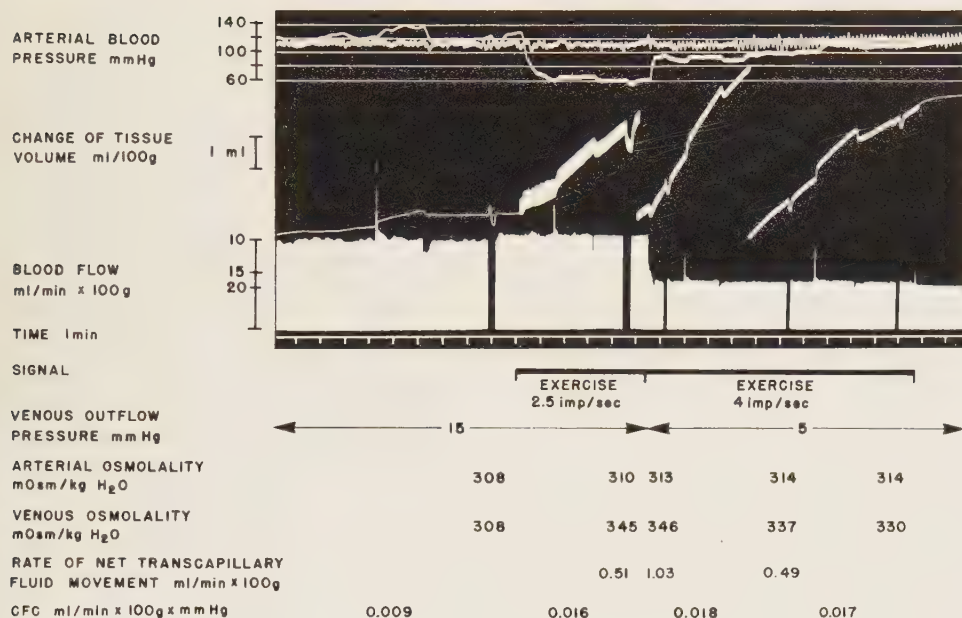


Fig. 23. Experiment indicating that the net transcapillary osmotic fluid flux to exercising muscle is proportional to blood flow under conditions of a constant veno-arterial osmolar difference. For explanation see text.

as was the induced veno-arterial osmolar difference. The fluid flux was thus directly proportional to the osmolar difference and therefore probably also to the transcapillary osmotic gradient (see above). The other experiments in this series supported this conclusion. These findings might further suggest that the substances responsible for tissue hyperosmolality at the different work intensities had roughly similar transcapillary reflection coefficients (see Comments).

Fig. 23 shows original tracings from a series of model experiments ($n = 4$) suggesting that the magnitude of the net transcapillary osmotic fluid flux to exercising muscle is directly related to the volume flow of blood under conditions of a constant veno-arterial osmolar difference. The osmotic fluid flux (tissue volume increase) was thus studied at two levels of blood flow, in this case 9.5 and 18 ml/min x 100 g tissue, flow being controlled by a perfusion pump. To be able to maintain an approximately constant veno-arterial osmolar difference at both these flow levels, the somato-motor fibre stimulation frequency had to be raised somewhat (from 2.5 to 4 imp/sec) during the larger flow. It is most likely that this had to be done to compensate for an increased "osmolar washout" from the tissue (decreased interstitial osmolality) and for a somewhat decreased transcapillary osmolar extraction. CFC during exercise was of similar magnitude at the two flow levels (Fig. 23) indicating that functional

capillary surface area and capillary flow distribution were kept approximately constant. Finally, significant variation of capillary hydrostatic pressure and thus of net fluid filtration at the two different blood flows was avoided by appropriate adjustments of venous outflow pressure. Thus, at the lower blood flow, venous outflow pressure was set at 15 mm Hg (in control state as well as during exercise at 2.5 imp/sec) and was lowered by 10 mm Hg when flow was increased in order to compensate for the concomitant rise of arterial inflow pressure of about 35 mm Hg. Assuming a ratio of pre- to post-capillary resistance of 3:1, mean hydrostatic capillary pressure can be calculated to be about 26 mm Hg at both flow rates. It can be seen that at the lower blood flow, exercise led to a net transcapillary fluid flux which, after the CFC determination, reached a maximal rate of $0.51 \text{ ml/min} \times 100 \text{ g tissue}$ at which time the veno-arterial osmolar difference was 35 mOsm. When flow was raised (approximately by 100 %) there was an abrupt increase of the rate of net transcapillary fluid movement to $1.03 \text{ ml/min} \times 100 \text{ g}$ which was then maintained constant for some minutes. The veno-arterial osmolar difference was almost the same as before (33 mOsm). The fact that a twofold increase of blood flow was associated with a twofold increase of the osmotic fluid flux suggests that the latter is directly related to volume flow of blood under conditions of constant veno-arterial osmolar difference. The other experiments in this series supported this conclusion; (Note in this connection that the results above indicated that the substances responsible for tissue hyperosmolality had similar transcapillary reflection coefficients at different stimulation rates).

In later phases of the work (Fig. 23) it can be seen that the net transcapillary fluid movement gradually declined with time. This can mainly be ascribed to a decreasing transcapillary osmolar gradient reflected in the lower veno-arterial osmolar difference (see below). Further, a gradual increase of tissue pressure could have contributed to this effect.

The experiments depicted in Figs. 22 and 23 thus indicate that the osmotic net transcapillary fluid movement during exercise may be directly related both to the veno-arterial osmolar difference and to blood flow. Therefore it would appear that in the individual exercise experiment the osmotic fluid flux is directly related to the product of veno-arterial osmolar difference and blood flow. This was analysed by plotting osmotic flux versus the above-mentioned product (Fig. 24) for all exercise experiments ($n = 60$) performed both when flow was free to change (average data previously discussed, Fig. 21) and when it was controlled with a perfusion pump (experiments exemplified in Figs. 22 and 23). The data were obtained after about 2—4 min of work of different intensities when the rate of transcapillary fluid movement was maximal. For the free flow experiments, osmotic fluid flux was calculated by subtracting net fluid filtration (capillary hydrostatic pressure increase $\times$ CFC) from the observed total fluid flux. It was assumed, as suggested in Chapter III, that capillary hydrostatic pressure was about unchanged during light exercise, and rose by 4 and 8 mm Hg

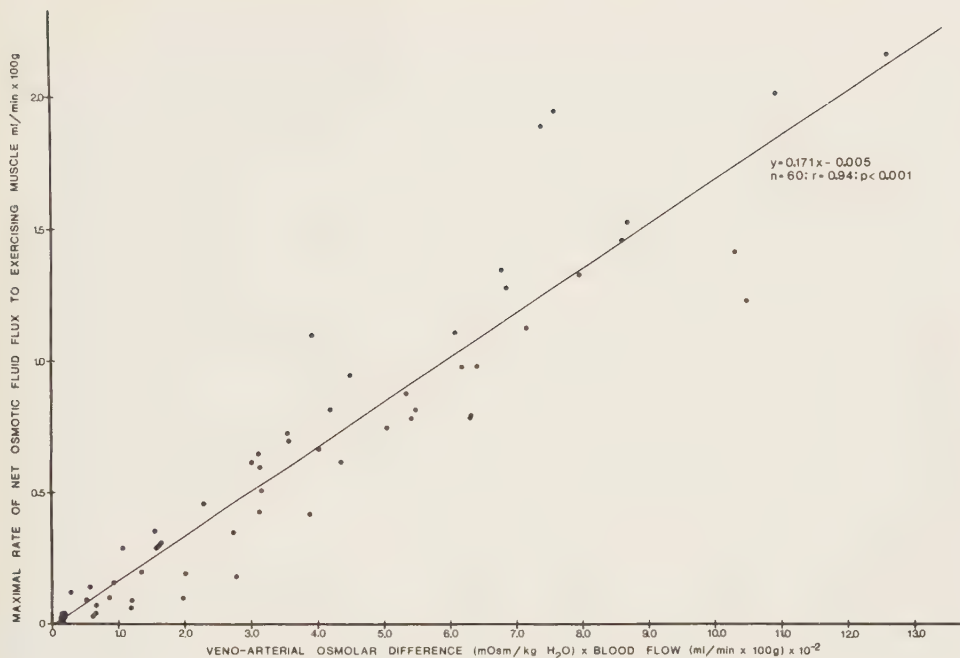


Fig. 24. Diagram of the maximal rate of net transcapillary osmotic fluid transfer into exercising muscle observed at different work intensities (sciatic nerve stimulation at 0.25–4 imp/sec) against the product of induced veno-arterial osmolar difference $\times$ blood flow $\times 10^{-2}$. Data indicate a direct relationship between the plotted parameters.

during moderate and heavy exercise respectively. (The other experiments were designed so that the osmotic fluid flux was obtained directly; see above). It can be seen from the diagram that the osmotic fluid flux was related to the calculated product and linear regression analysis indicated a direct relation ($r = 0.94$, $p < 0.001$). The regression line intersects the vertical axis at a point near the origin. Spread of data in the diagram might be entirely due to difficulties in determining the measured variables exactly. A non-linearity, however, should be present if solutes with different transcapillary reflection coefficients were responsible for the interstitial hyperosmolality at different work intensities. This possibility seemed less likely, however, since the osmotic fluid flux per unit veno-arterial difference $\times$ flow showed no statistically significant correlation to the rate of somato-motor fibre stimulation, an observation which supported the earlier conclusion reached from the type of experiment described in Fig. 22. That indeed a linear relation between the variables in Fig. 24 should be present is supported by the analysis given in Comments section. The regression equation found in Fig. 24 will be used in an attempt to distinguish the hydrostatic and osmotic forces involved in the fluid accumulation in muscle during prolonged work and in the disappearance of accumulated fluid in the postexercise period. These problems will be considered in the following sections.

During "prolonged" exercise periods (15 min), the hyperemia (vascular conductance) and the CFC usually remained approximately constant of the levels observed in the early phase (<4 min). Sometimes, however, CFC determinations performed in late periods of prolonged heavy work showed somewhat lower values than in early periods, but this may merely reflect interference on the determination from a gradual increase of tissue pressure (*cf.* Jacobsson and Kjellmer 1964). The rate of net transcapillary fluid movement was relatively constant throughout light exercise of 15 min duration but it declined gradually with time during heavy exercise. These findings may mainly be explained by the fact that the transcapillary osmotic gradient, as reflected by the veno-arterial osmolar difference, remained approximately constant during light exercise, but showed a gradual decrease during heavy work (*cf.* Fig. 23). Thus, the arterial osmolality as well as the venous hyperosmolality remained roughly constant throughout light exercise whereas, during heavy exercise, arterial osmolality tended to increase and venous hyperosmolality tended to decrease with time (*cf.* Chapter II, Results section A 1).

Fig. 25 shows collected mean data $\pm$ SEM ($n=9$) on fluid accumulation (left ordinate) in muscle during prolonged heavy exercise and on the evoked changes of the veno-arterial osmolar difference (right ordinate), the latter determined 3, 7 and 15 min after the commencement of work; (correction was here made for the initial capacitance response which averaged 0.58 ml/100 g tissue). It can be seen that the rate of transcapillary fluid movement was maximal in the time period some 1 to 4 min after beginning of work and that it then decreased gradually. After 15 min of work, the total amount of accumulated fluid averaged 14.8 ml/100 g tissue. The induced veno-arterial osmolar difference averaged 17.0 mOsm after 3 min, 9.4 mOsm after 7 min, and 3.3 mOsm after 15 min of work. These data strongly suggest that the gradual decline of the rate of fluid accumulation in the exercising muscles to a great extent can be ascribed to the successive decrease of the veno-arterial osmolar difference (reflecting the transcapillary osmolar gradient).

It is conceivable, however, that the pronounced increase of extravascular fluid volume could lead to some increase of tissue pressure which also would tend to counteract the transcapillary fluid flux. The following deductions may permit a rough estimation of the extent to which tissue pressure changed, on the average, in the experiments depicted in Fig. 25. CFC averaged 0.037 ml/min $\times$ 100 g $\times$ mm Hg in early phases of work. Assuming, as discussed above, that mean capillary hydrostatic pressure during heavy exercise rises by 8 mm Hg, the resulting net fluid filtration at 3 min of exercise would amount to 0.30 ml/min $\times$ 100 g, if no significant tissue pressure changes were present at this time. The average rate of total fluid flux at 3 min was 1.92 ml/min $\times$ 100 g (calculated as the mean of the fluid flux during the 3rd and 4th min) and the osmotic fluid flux therefore was 1.62 ml/min $\times$ 100 g. The latter figure agrees well with the osmotic flux deduced from the regression line of Fig. 24 which showed the

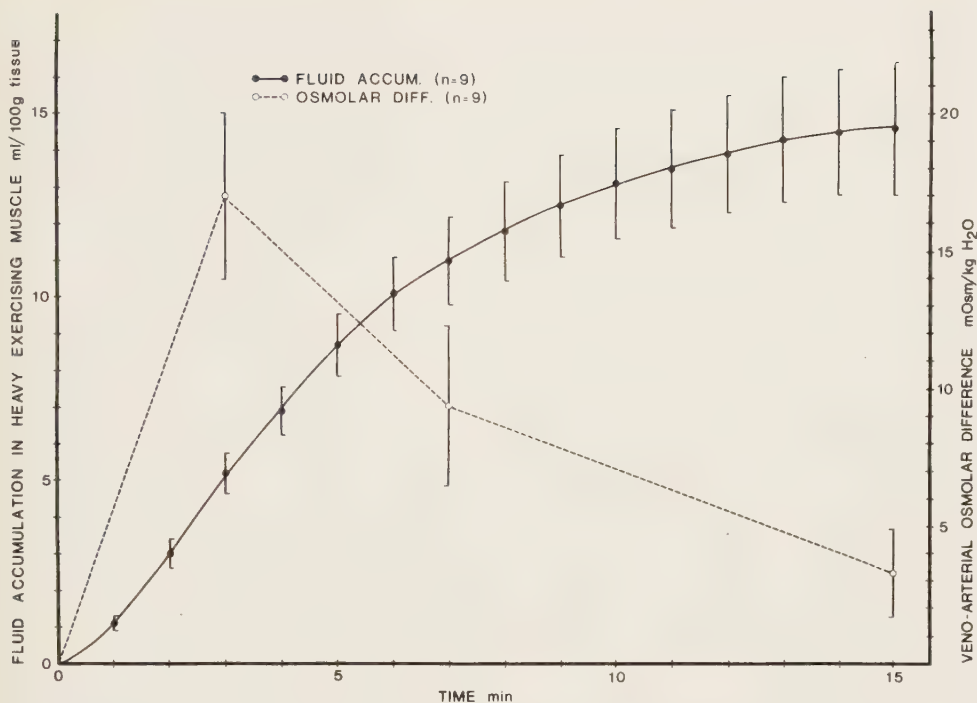


Fig. 25. Diagram showing mean data $\pm$ SEM (9 exp.) on the fluid accumulation in skeletal muscle (left ordinate) during 15 min of "heavy" exercise (somato-motor fibre stimulation 4 imp/sec) and on the concomitant work induced veno-arterial osmolar difference (right ordinate), the latter determined 3, 7, and 15 min after commencement of work.

relation between the osmotic fluid flux and the product of veno-arterial osmolar difference times blood flow for short-term exercise. Thus this product was 8.98×10^2 at 3 min (blood flow averaged 52.8 ml/min $\times$ 100 g) giving a value for osmotic fluid flux of 1.53 ml/min $\times$ 100 g. If a linear relationship between the osmotic fluid flux and this product (P) were present throughout prolonged work the osmotic fluid flux at 7 min (blood flow averaged 43.7 ml) can be calculated as $(P \text{ 7 min}/P \text{ 3 min}) \times \text{osmotic fluid flux 3 min}$. This gives a value of 0.74 ml/min $\times$ 100 g $((4.11 \times 10^2/8.98 \times 10^2) \times 1.62)$. A similar deduction for osmotic fluid flux at 15 min (blood flow averaged 38.8) gives a value of 0.23 ml/min $\times$ 100 g $((1.28 \times 10^2/8.98 \times 10^2) \times 1.62)$; (it may be mentioned that the somewhat reduced hyperemia with time during the exercise largely could be attributed to a decrease of the perfusion pressure). The capillary hydrostatic pressure rise and CFC should be roughly similar throughout the prolonged exercise and the rate of net transcapillary fluid filtration, in turn, should therefore be about constant, or 0.30 ml/min $\times$ 100 g, if no change of tissue pressure occurred. Theoretically therefore, the rate of total fluid flux at 7 and 15

min of work should be $1.04 (0.74 + 0.30)$ and $0.53 (0.23 + 0.30)$ ml/min $\times$ 100 g, respectively. The observed rates of total fluid flux in these periods, however, averaged 0.84 (mean of fluid flux during the 7th and 8th min) and 0.13 ml/min $\times$ 100 g (fluid flux during the 15th min), *i.e.* 0.20 and 0.40 ml/min $\times$ 100 g less than that theoretically expected if there were no change of tissue pressure. A value for the increase of tissue pressure may be obtained by dividing these latter values by CFC ($0.20/0.037$, $0.40/0.037$) thus indicating that tissue pressure had increased by 5 mm Hg at 7 min and by 11 mm Hg at 15 min of exercise. Admittedly, these calculations are crude and indirect but nevertheless the figures for the tissue pressure change, after correction for the effects of some increase of plasma colloid osmotic pressure, are in close agreement with reported findings obtained with an independent method (see Comments).

After cessation of exercise, tissue volume slowly and gradually decreased towards the resting control level prevailing before work. The time required for this return of tissue volume was related to the amount of accumulated fluid at the end of the exercise period. After prolonged (15 min) heavy exercise, when the fluid accumulation could exceed 20 ml/100 g tissue, tissue volume was sometimes not restored until 4 hours had elapsed. The rate of tissue volume decline was most rapid in the initial period after the cessation of exercise and decreased with time.

In an attempt to analyse the mechanisms responsible for the fluid absorption after exercise, tissue volume, blood flow, CFC, and arterial and venous osmolality were followed after prolonged (10–15 min) heavy work (6 animals). The mean results $\pm$ SEM are shown in Fig. 26. In the diagram, the disappearance of the fluid accumulated in the muscles during exercise (left ordinate) and the arterio-venous osmolar difference (right ordinate) in the post-exercise period are plotted against time. Other, calculated, variables are shown below the diagram. It can be seen that the accumulated fluid, which averaged 14.8 ml/100 g at the end of the exercise period, disappeared at gradually declining rates over the time period of 3 hr, at the end of which there was still some of the accumulated fluid present (3 ml/100 g tissue).

A constant observation was that the veno-arterial osmolar difference present during work (see Fig. 25) was reversed in the post-exercise period implying that after exercise arterial osmolality in fact exceeded venous osmolality (*i.e.* there was a transcapillary osmolar gradient from blood to tissue). As can be seen from Fig. 26 the average negative arterio-venous osmolar difference of 7 mOsm at the end of work was reversed already 5 min after the cessation of exercise to a positive value of some 2 mOsm. The arterio-venous osmolar difference gradually rose to a plateau-value of about 4 mOsm in a time of between 20 — 80 min after which it again declined. The reversal of the arterio-venous difference after work was due to declining venous osmolality, whereas the arterial osmolality which was raised during the prolonged work remained elevated for a considerable length of time after exercise. It is conceivable that the higher blood

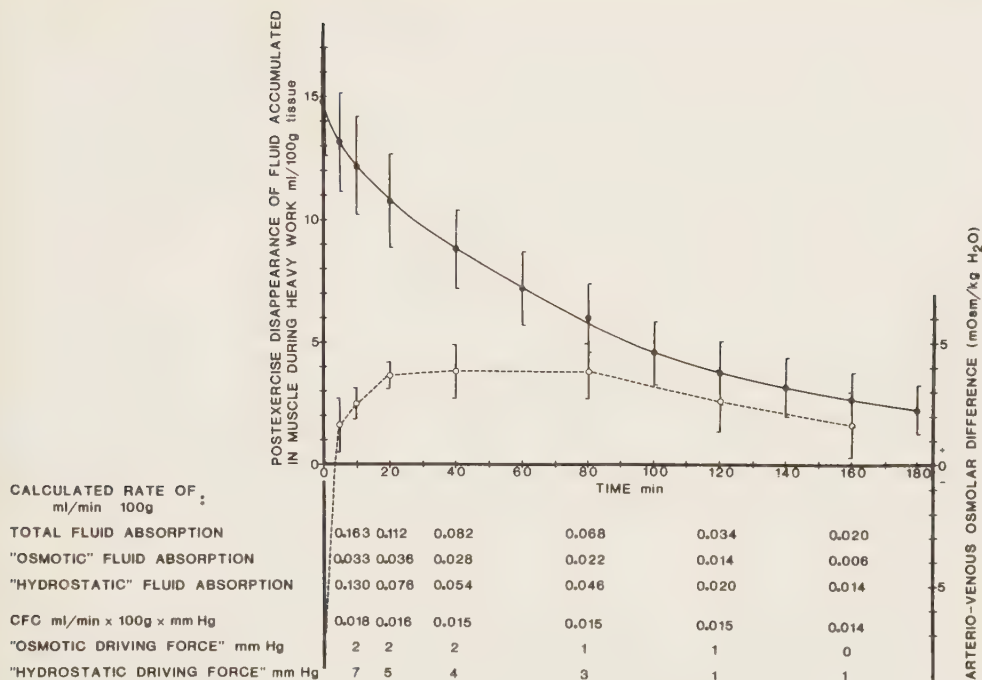


Fig. 26. Diagram showing mean data $\pm$ SEM (6 exp.) on post-exercise disappearance of fluid accumulated in muscle during work (left ordinate) and of the concomitantly observed arterio-venous osmolar difference (right ordinate). Derived variables shown at bottom of figure.

than tissue osmolality in the post-exercise period could cause an osmotic fluid absorption and partly explain the fluid disappearance; (Note that in these experiments lymph drainage was prevented implying that fluid disappearance solely occurred as a transcapillary process). It seems that the importance of an osmotic fluid flux, caused by solutes other than plasma proteins, might be revealed from values for blood flow and arterio-venous osmolar difference if a linear relationship between the product of these two factors and osmotic fluid flux (like in Fig. 24) could be established also with regard to transcapillary fluid absorption. That this is so is strongly suggested by the data given in Fig. 27 which is based on results obtained during intra-arterial hypertonic glucose infusion to the resting skeletal muscle. The data refer to constant pressure perfusion experiments and the hypertonic infusions were adjusted so as to elicit varying rates of fluid absorption and hyperemia responses. (A possible decrease of tissue pressure was considered roughly balanced by the concomitant rise of capillary hydrostatic pressure during hypertonic infusion, *cf.* Chapter III). It can be seen that the equations for the regression lines in this figure and in Fig. 24 are quite similar. Calculation of osmotic fluid absorption from either equation would thus give similar results. That of Fig. 24 is used below because its regression line, as should be expected, passes through the origin (or nearly so)

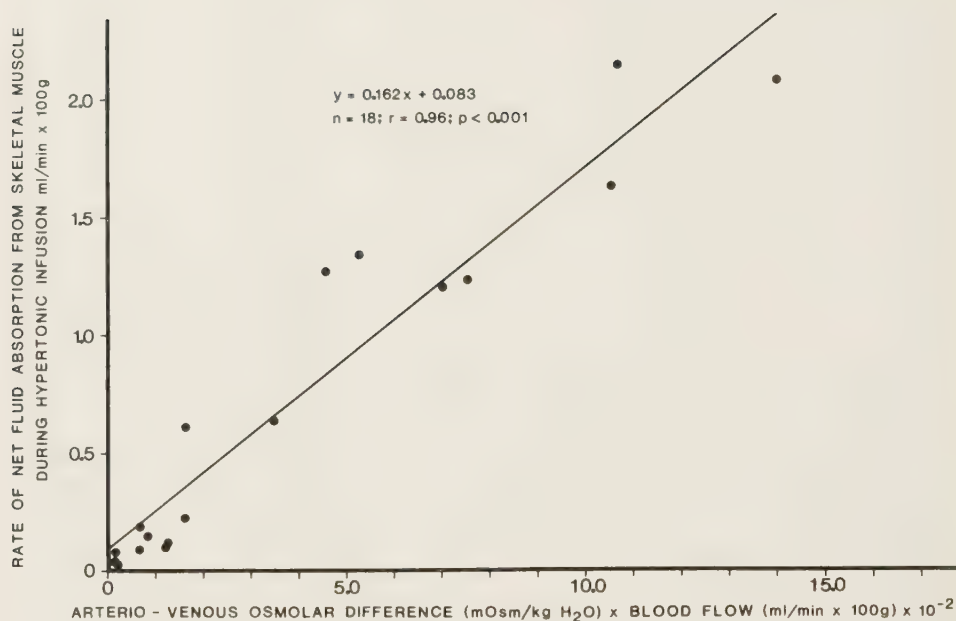


Fig. 27. Rate of net transcapillary fluid absorption in skeletal muscle caused by i. a. infusion of hypertonic glucose solution plotted against the product of the simultaneously determined arterio-venous osmolar difference $\times$ blood flow $\times 10^{-2}$. A direct relation seems present.

and because it represents osmotic flux caused by solutes present in the exercise and probably also in the post-exercise period (see Comments).

The data presented below the diagram in Fig. 26 shows for the post-exercise period the following deduced variables: The rates of total fluid absorption (obtained from tangents drawn to the smooth absorption curve), of osmotic fluid absorption (obtained from the depicted average values for arterio-venous osmolar difference and from average blood flow values using the regression equation in Fig. 24), and of fluid absorption caused by transcapillary "hydrostatic" (including colloid osmotic) forces (difference between total and osmotic fluid absorption); further, observed mean CFC values and calculated transcapillary "driving forces" are shown. Data on fluid absorption rates are given at intervals from the 10th min after cessation of work. At this time the post-exercise hyperemia, and therefore the co-ordinated regional blood volume change, had disappeared which implied that the tissue volume decline after this period could be entirely ascribed to net transcapillary fluid movement. The calculated data in Fig. 26 suggest that the fluid absorption was mainly due to transcapillary hydrostatic and colloid osmotic forces; osmotic absorption caused by smaller

molecules might also be quite significant, being responsible for 20 to 40 per cent of total fluid absorption. By dividing the calculated rates of transcapillary fluid absorption by the CFC, the depicted values for effective transcapillary "hydrostatic and osmotic" driving forces were obtained. Note that the early value for hydrostatic force agrees quite well with the values for "tissue pressure" increase during exercise arrived at in Fig. 25.

COMMENTS

The aim of this study was to analyse quantitatively the net transcapillary fluid movement in skeletal muscle during and after exercise and to elucidate the mechanisms responsible for this transport. To be able to study transcapillary events only, possible lymph drainage had to be excluded. This was done by ligation and cauterization of the regional lymph vessels. The significance of such interference with lymph drainage for the fluid accumulation and disappearance during and after work will be considered later on.

As early as 1915, Barcroft and Kato postulated from indirect measurements that the transcapillary fluid flux into exercising muscle must be ascribed not only to increased capillary hydrostatic pressure but also to osmosis due to metabolically increased tissue osmolality. This opinion received further support from later measurements of regional changes of vapor pressure during exercise (for details see review by Landis 1934). On the other hand, in a recent study (Kjellmer 1964 *a*), in which transcapillary fluid movement into exercising muscle was measured more directly, it was concluded that the fluid transfer was caused solely by filtration due to an increased capillary hydrostatic pressure. This conclusion was mainly based on the finding that mechanical reduction of arterial inflow pressure, and hence of capillary pressure, could lead to abolition of the transcapillary fluid flux in exercising muscle. Since this procedure, in addition, caused pronounced reduction of regional blood flow, and since the present results indicate that osmotic fluid flux in exercise is flow dependent (see further below), the osmotic mechanism for fluid transfer might easily have been overlooked in that type of experiment.

In the present study, as in that of Kjellmer, net transcapillary fluid movement into muscle was followed by continuous volumetric recording of changes in tissue volume. With this technique it is possible to distinguish between alterations of intra- and extravascular fluid volumes (see Methods). From the present investigation of concomitantly occurring fluid transfer, changes of capillary hydrostatic pressure, transcapillary hydrodynamic conductivity (CFC) and regional osmolar events it seems clear that the fluid accumulation in exercising muscle is mainly due to transcapillary fluid osmosis resulting from regional tissue hyperosmolality. Thus, at all work intensities, more than 75 per cent of the transcapillary fluid flux could be ascribed to fluid osmosis, the remaining part being due to fluid filtration caused by increased capillary hydrostatic pressure (e.g. Fig. 21). This estimation was derived from analyses performed in the phase of maximal

fluid transfer rate occurring early in exercise but appears representative also for later stages of work (as can be deduced from the data in Fig. 25). To exemplify that the fluid flux can by no means be explained by filtration alone, it may be mentioned that the fluid transfer sometimes was so rapid as to require a rise of mean capillary hydrostatic pressure of nearly 100 mm Hg (*e.g.* panel A, Fig. 20). This value far exceeds the capillary pressure rise that can occur in exercising muscle at heart level. As indicated by the present results, the capillary pressure increase at most can amount to 10 to 15 mm Hg. The only reasonable conclusion is that the pronounced tissue hyperosmolality is responsible for the major part of the fluid transfer.

During prolonged work (15 min duration) the net transcapillary fluid movement tended to decline gradually with time, a feature most prominent during heavy work (Fig. 25). This decline could be ascribed mainly to a successive decrease of the transcapillary osmolar gradient, due partly to some increase of arterial osmolality and partly to a decrease of the interstitial (venous) hyperosmolality. To some extent the declining rate of fluid flux seemed also related to a gradual increase of tissue pressure. The increase of arterial osmolality can be explained, at least partly, by a "delivery of osmols" from the exercising muscles to the general circulation, and the decline of interstitial hyperosmolality partly by a possible decrease of the "osmolar production" in the active muscles and partly by a regional "osmotic dilution" as tissue fluid volume increases.

The magnitude of the tissue pressure changes caused by the fluid accumulation in exercising cat calf muscles has previously been evaluated by Kjellmer (1964 *b*). Since tissue pressure seemed to vary between different portions of the muscles his data were given in terms of a minimal and a maximal pressure value. An increase of extravascular tissue volume by 10 to 15 per cent caused tissue pressure to rise by some 3 to 6 mm Hg (deduced from Fig. 7 in Kjellmer's paper). Rough deductions from the present data (Fig. 25) suggested that "tissue pressure" rose by some 5 to 11 mm Hg at a comparable increase of tissue volume (10 to 15 per cent), but this estimation also included the effect of a possibly altered transcapillary colloid osmotic pressure gradient. The effect of changes of the interstitial protein concentration during exercise would appear almost negligible, since the resting protein concentration is low and since a significant part of the fluid transferred into the working muscles apparently accumulates inside the striated muscle cells (see below). The effect of altered plasma protein concentration, on the other hand, may be significant due to the relatively high concentration at rest and to the disproportionate increase of plasma colloid osmotic pressure as a function of plasma fluid loss (see Landis and Pappenheimer 1963). From the observed plasma fluid loss it can thus be calculated that plasma colloid osmotic pressure on an average might have risen by about 2 mm Hg towards the end of the exercise experiments depicted in Fig. 25. The present data may therefore be taken to indicate a tissue pressure increase during prolonged exercise of some 3 to 9 mm Hg, which is relatively close to Kjellmer's

findings (1964 *b*). Mention should finally be made that when resting skeletal muscle volume was increased by 15 per cent, Guyton (1965) found tissue pressure to rise by about 6 mm Hg from a negative value in the control period.

The total amount of fluid that accumulated in the cat lower leg muscles during 15 min of heavy work (4 imp/sec) averaged, as mentioned above, no less than about 15 ml/100 g tissue (Fig. 25), which is in agreement with the highest values reported by Jacobsson and Kjellmer (1964). More than 60 per cent of this "oedema" was formed within the first 5 min of work. Under normal *in vivo* conditions, the fluid accumulation would be less pronounced due to fluid drainage *via* the intact lymph system. Jacobsson and Kjellmer (1964) who studied the cumulative outflow of lymph from a catheterized popliteal main lymph vessel in the cat during a 20 min period of work of the calf muscles found, however, that it was quite small, and no more than a total of 0.1 ml. Since other lymph vessels in the knee region were ligated in that study, total lymph drainage may be somewhat larger in the intact organism. Nevertheless, these data suggest that lymph drainage from working muscle is quite small and, hence, that the fluid accumulation in the active muscle may not be much less under normal circumstances than found in the present study. Perhaps the surprisingly small lymph flow is explained by the fact that a considerable part of the fluid transferred from the blood stream must accumulate in the intracellular space of muscle (see below).

It should be stressed that the present data on fluid accumulation in muscle refer to work performed with a relatively small part of the total muscle mass of the animal. In situations where the major part of the muscle mass in the body is active, the fluid accumulation per unit tissue must be far less since it can be calculated that, otherwise, virtually all plasma fluid would be lost within some 5 min of heavy work. Under these circumstances, mechanisms which limit the plasma fluid loss must therefore be put into play. This problem, which was approached in a recent study (Lundvall *et al.* 1970, 1972), will be discussed below (General discussion).

The tissue hyperosmolality in exercising muscle must primarily be created inside the contracting striated muscle cells as a result of the increased tissue metabolism. A significant, perhaps major, part of the osmotically transferred fluid from the blood stream can therefore, as already mentioned, be expected to accumulate in the intracellular space of striated muscle. This opinion is supported by a recent study by Bergström *et al.* (1971) who estimated changes of extra- and intracellular water content in muscle evoked by prolonged (20 min) moderate to heavy work in man. On the average, total muscle water, calculated in ml/100 g fat-free solids, increased by 21 per cent and the increased water content was estimated to be roughly equally distributed between the intracellular and interstitial spaces.

The fluid volume that accumulates in the skeletal muscle during exercise, regardless of its final distribution into the different tissue compartments, is of

course derived from the blood stream and therefore this entire fluid volume must traverse the capillary membranes. Some characteristics of this capillary exchange process will be considered below.

It is generally believed that plasma fluid passes the capillary barrier *via* gaps (about 40 Å "pores") at the intercellular (endothelial) junctions but, as suggested by recent findings in heart muscle, water may also to some extent traverse the capillary endothelial cells themselves (Yudilevich and Alvarez 1967). However, the quantitative relation between fluid movements *via* these routes is so far unknown.

Evidence was presented above that the fluid flux to the extravascular space of exercising muscle was partly due to a filtration process and partly to an osmotic process. Net fluid filtration into the tissue occurs at a rate determined by the product of the increase of the transcapillary hydrostatic pressure gradient and the prevailing transcapillary hydrodynamic conductivity; the latter being measured for the whole region in terms of CFC in the present study. Data on these quantities were presented above and, in addition, a rough evaluation of secondary changes of the transcapillary colloid osmotic pressure gradient was made. The osmotic transcapillary fluid flux into the exercising muscle (primarily into its interstitium) is a more complex process influenced by a multitude of factors. The results presented in Chapter II indicated that the interstitial hyperosmolality in exercise can be ascribed to increased concentrations of several substances, in early phases especially of sodium and lactate, but also of others such as potassium etc. The increased concentrations of most of these agents in the interstitium would seem mainly to be caused by release of osmotically active products from the contracting muscle fibres; the osmotic transfer of water into the intracellular (hypertonic) space of muscle must also contribute to raised interstitial solute concentrations (e.g. with regard to sodium). The evoked osmotic transcapillary fluid flux should be governed, among other things, by the transcapillary concentration (osmolar) gradients for these different substances, by their transcapillary osmotic reflection coefficients, and by the capillary hydrodynamic conductivity. The present study does not, of course, permit an assessment of the complex interplay between all individual factors determining the osmotic fluid flux during exercise but nevertheless, the present analysis (see Figs. 22—24) may lead to some conclusions about certain basic characteristics of this process.

Before discussing these results, some factors of importance for the capillary exchange will be considered. CFC is a measure of the total transcapillary hydrodynamic conductivity in the region and is, in turn, a function of the specific capillary permeability (per unit area) and the prevailing capillary surface area available for fluid exchange (e.g. Folkow and Mellander 1970). Since there is no indication that capillary permeability in muscle is altered by exercise (Arthurson and Kjellmer 1964), changes of CFC would reflect alterations of the functional capillary surface area, as caused by changes of the number of patent

(perfused) capillaries and controlled by the activity of the precapillary "sphincters" (*cf.* Mellander and Johansson 1968). Although the bore and length and thus the surface area of individual capillaries vary considerably, differences in CFC will provide information about changes in the number of patent, "average" capillaries because of the inclusion of a vast number of capillaries on a random basis in every measurement. It follows that relative changes of capillary flow velocity will be reflected by changes in the ratio of blood flow/CFC.

Experimental evidence (Fig. 22) indicated that the osmotic fluid flux was directly proportional to the veno-arterial osmolar difference (varied by performing work at different intensities) under conditions when blood flow and CFC were kept constant and therefore, capillary surface area, capillary flow distribution, capillary flow velocity, and capillary permeability could be considered constant (*cf.* also Lundgren and Mellander 1967). Further, under conditions of approximately constant veno-arterial osmolar difference and CFC, the osmotic fluid flux was directly proportional to the volume flow of blood (Fig. 23) and, hence, to capillary flow velocity. The collected data from exercise experiments of different intensities (light to heavy work) in which blood flow, CFC and veno-arterial osmolar difference varied within wide ranges, indicated that the osmotic fluid flux was directly proportional to the product of the veno-arterial osmolar difference and volume flow of blood (Fig. 24).

The interpretation of the present results may be aided by the theoretical analysis below although this analysis certainly implies that the osmotic fluid exchange process is viewed in a simplified way. For this analysis the following assumptions were made: 1) The hyperosmolality in the interstitium of the exercising muscle is considered uniform (well mixed tissue compartment) without any significant osmolar gradient along the external side of the capillary. This assumption may be justified for several reasons. All muscle fibres were contracting (supramaximal somato-motor fibre stimulation) thus "delivering osmols" throughout the interstitium. The extensive anastomosing in the capillary net work (*e.g.* Wiedeman 1963) tends to minimize interstitial solute concentration gradients. Further, the fluid streaming into the interstitial space during work might have a "stirring" effect (*cf.* Lundgren and Mellander 1967). 2) Solute and fluid permeability is considered roughly constant along the capillary. The longitudinal gradient of capillary permeability observed in some tissues (*e.g.* Zweifach and Intaglietta 1968) has so far not been clearly demonstrated in skeletal muscle (Smaje *et al.* 1970).

Fig. 28 depicts the osmolality increase in blood passing a single idealized "unit" capillary in exercising muscle. L denotes the total length of the capillary and x distance from its orifice on the arterial side; $\Delta\text{Osm}(0)$ denotes the osmolality difference between the interstitial space and the blood at the arterial orifice, and $\Delta\text{Osm}(x)$ the osmolar difference at distance x from the orifice. $\Delta\text{Osm}(V-A)$ denotes the measured veno-arterial osmolar difference. For a stagnant column of capillary blood, the decrease of the transcapillary osmolar difference, caused

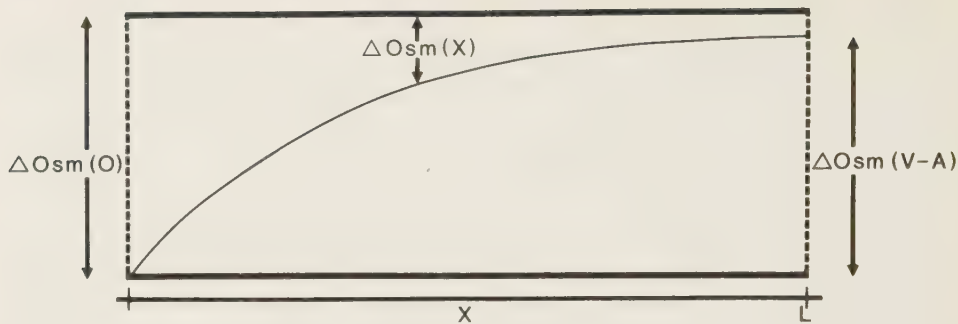


Fig. 28. Schematic illustration of osmolality increase in blood passing a single idealized "unit" capillary in exercising muscle. L denotes the total length of the capillary and x distance from the orifice on the arterial side. $\Delta \text{Osm}(0)$ denotes the osmolality difference between the interstitial space and blood at the arterial orifice and $\Delta \text{Osm}(x)$ the osmolality difference at distance x . $\Delta \text{Osm}(V-A)$ denotes determined veno-arterial osmolar difference.

by solute diffusion from the interstitial space and fluid transfer in the opposite direction, may be described by the differential equation:

$$\frac{d(\Delta \text{Osm}(t))}{dt} = -\frac{\Delta \text{Osm}(t)}{\tau} \quad (1)$$

where τ is a time constant determined by the characteristics of the substances involved in the exchange process, capillary pore size, temperature etc. The solution to equation (1) is:

$$\Delta \text{Osm}(t) = \Delta \text{Osm}(0) \cdot e^{-\frac{t}{\tau}} \quad (2)$$

If blood moves through the capillary with a constant velocity, v , equation (2) yields

$$\Delta \text{Osm}(x) = \Delta \text{Osm}(0) \cdot e^{-\frac{x}{v \cdot \tau}} \quad (3)$$

where $\tau = \frac{x}{v}$, i.e. distance/(distance/time).

By integration over the entire length of the capillary, the mean transcapillary osmolar difference is obtained.

$$\langle \Delta \text{Osm}(x) \rangle = \frac{1}{L} \int_0^L \Delta \text{Osm}(x) dx = \Delta \text{Osm}(0) \cdot \frac{v \cdot \tau}{L} (1 - e^{-\frac{L}{v \cdot \tau}}) \quad (4)$$

Considering the muscle capillary bed as consisting of a number of "unit" capillaries with an overall transcapillary hydrodynamic conductivity expressed by CFC, Van't Hoff's law, modified with regard to the osmotic reflection coefficient for the case of leaky capillary membranes (*cf.* Landis and Pappenheimer

1963), gives the following expression of the osmotic transcapillary fluid flux, J , into exercising muscle in a given situation:

$$J = R \cdot T \cdot \Delta \text{Osm}(0) \cdot \frac{v \cdot \tau}{L} \cdot \left(1 - e^{-\frac{L}{v \cdot \tau}} \right) \cdot \sigma \cdot \text{CFC} \quad (5)$$

In this formula J and CFC are expressed per unit muscle weight, and σ represents a "weighed mean" of the transcapillary osmotic reflection coefficients for the substances responsible for the interstitial hyperosmolality of exercise; (R but also T and L are constants).

In the present experiments, $\Delta \text{Osm}(0)$ could not be measured and the osmotic fluid flux was studied merely in relation to the veno-arterial osmolar difference, $\Delta \text{Osm}(V-A)$, which, according to (3), can be written:

$$\Delta \text{Osm}(V-A) = \Delta \text{Osm}(0) - \Delta \text{Osm}(0) \cdot e^{-\frac{L}{v \cdot \tau}} = \Delta \text{Osm}(0) \left(1 - e^{-\frac{L}{v \cdot \tau}} \right) \quad (6)$$

Equation (5) thus be transformed:

$$J = R \cdot T \cdot \Delta \text{Osm}(V-A) \cdot \frac{v \cdot \tau}{L} \cdot \sigma \cdot \text{CFC} \quad (7)$$

The results presented in Figs. 22 to 24 may now be discussed in view of this latter expression. In the experiments illustrated in Fig. 22, blood flow and CFC and therefore also v (proportional to flow/ CFC , see above), were kept constant. J was found to vary in direct relation to $\Delta \text{Osm}(V-A)$, changes of the latter being produced by varying the sciatic nerve stimulation frequency (work intensity). Since R , T and L are constant it follows that the product $\sigma \cdot \tau$ can be considered constant. Thus, at different work intensities, both σ and τ either were constant, or they changed equally much in opposite directions. If σ increased with increasing work intensity it would imply that the solutes responsible for the interstitial hyperosmolality exhibited a corresponding decrease of their transcapillary diffusibility. According to (1), however, τ must then increase concomitantly, unless the fluid transferred in the osmolar exchange process, as a result of a simultaneous change of its tonicity, contributed significantly more to the osmolar equilibration between tissue and blood than at low work intensities, which appears less likely. The reverse would be true if σ decreased. The experimental findings, as already mentioned, seemed to refute the possibility that σ and τ change in the *same* direction. It may be concluded that σ as well as τ tended to be roughly constant at different work intensities. According to (6) and (4) the veno-arterial osmolar difference, in its turn, would then be proportional to the mean transcapillary osmolar difference under the conditions prevailing in the experiment of Fig. 22.

In Fig. 23, $\Delta \text{Osm}(V-A)$ and CFC were kept roughly constant while blood flow was varied. Thus v varied with blood flow and, according to (7), as did J . It is clear that in this type of experiment $\Delta \text{Osm}(0)$ increased with v (see(3)).

The data in Fig. 24, finally, indicated that J varied directly with the product blood flow times $\Delta \text{Osm}(V-A)$. This product can be written as

$\frac{\text{flow}}{\text{CFC}} \cdot \text{CFC} \cdot \Delta \text{Osm}(V-A)$, or, since $\frac{\text{flow}}{\text{CFC}}$ is proportional to v , as a constant $\cdot v \cdot \text{CFC} \cdot \Delta \text{Osm}(V-A)$. It follows that the linear relationship indicated by the experimental data depicted in Fig. 24 is in accordance with (7).

An attempt will be made below to estimate to what extent venous hyperosmolality reflects interstitial hyperosmolality induced by work, a problem of great importance for the quantitative evaluation of the role of hyperosmolality in exercise hyperemia. Another approach to the same problem was presented in Chapter II. The relation between venous and interstitial hyperosmolality, using the above defined terms, is given by the ratio of $\Delta \text{Osm}(V-A)/\Delta \text{Osm}(0)$

which, in turn, can be expressed as $\Delta \text{Osm}(0) \cdot (1 - e^{-\frac{L}{v \cdot \tau}}) / \Delta \text{Osm}(0)$, or

$(1 - e^{-\frac{L}{v \cdot \tau}})$ (see (6)). This expression can be derived from (7) by using the experimentally observed values for J , CFC, and $\Delta \text{Osm}(V-A)$ and given values for σ . σ , as mentioned above, is here considered as the weighed mean of the individual transcapillary reflection coefficients for the solutes responsible for the work induced interstitial hyperosmolality (mainly sodium and lactate, but also others, such as potassium etc; Chapter II).

Since σ could not be determined in the present experiments seemingly reasonable σ values were taken from the literature. Vargas and Johnson (1964) presented experimental evidence for heart capillaries to suggest that the reflection coefficients for small lipid insoluble molecules are far below unity, for urea 0.10 and for sucrose 0.30. However, these results did not receive unanimous support (e.g. Pappenheimer 1970) since they seemed too low to be compatible with the concept of the "pore" dimension established by extensive studies in skeletal muscle. Yet, later theoretical calculations by Perl (1971) who took the special geometry of the capillary pores (Karnovsky 1967, 1970) and the possible fluid transfer across the capillary endothelial cells (Yudilevich and Alvarez 1967) into account, gave values for σ in skeletal muscle capillaries similar to those found by Vargas and Johnson. Perl thus arrived at a value for σ for NaCl of 0.16—0.18, for urea of 0.21—0.23, and for glucose of 0.22—0.27. Interestingly, this analysis led to the view that such low reflection coefficient values still were in consonance with the classical estimates of capillary "pore" size (see Landis and Pappenheimer 1963).

For the present analysis, dealing with exercise hyperosmolality created by small molecules, the weighed mean of the transcapillary osmotic reflection coefficients, in view of the discussed data, was considered to be in the range of 0.10 to 0.30. In the diagram of Fig. 29 the ratio of $\Delta \text{Osm}(V-A)/\Delta \text{Osm}(0)$ is plotted against the ratio of blood flow/CFC. From what has been said the dia-

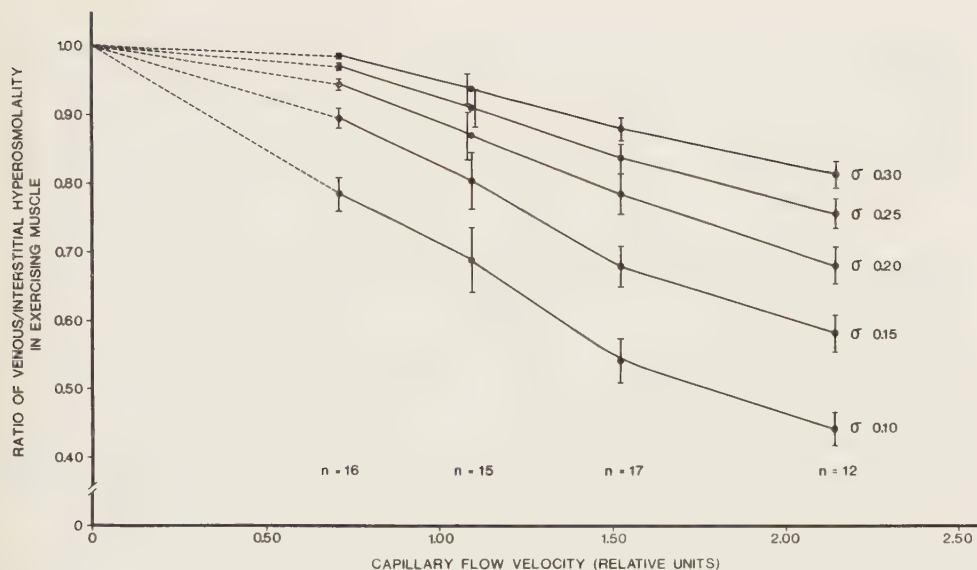


Fig. 29. Ratio of venous/interstitial hyperosmolality in exercising muscle against (blood flow/CFC) $\times 10^{-3}$. The latter expression used as a relative measure of capillary flow velocity. The hyperosmolality ratio is given for various values for transcapillary osmotic reflection coefficient, σ ("weighed mean" for the substances responsible for the interstitial hyperosmolality of exercise).

gram thus shows the extent to which work induced interstitial hyperosmolality is reflected in the venous effluent ("hyperosmolar extraction") at different capillary flow velocities expressed in relative units. The diagram shows classed mean values $\pm$ SEM for 4 ranges of flow/CFC (<0.90 , mean 0.71 ± 0.04 (SEM); $0.90-1.30$, mean 1.09 ± 0.03 ; $1.30-2.00$, mean 1.52 ± 0.05 ; and >2.00 , mean 2.14 ± 0.18 . All flow/CFC values have been multiplied by 10^{-3}). The data indicate that for any σ value the hyperosmolar extraction is clearly below unity at the higher capillary flow rates. Note that "extraction" varies considerably with σ , which for the present purpose implies that σ has to be more closely defined. An idea of the magnitude of σ can be obtained by comparing the regression lines in Fig. 24 (exercise experiments) and Fig. 27 (hypertonic glucose infusion). The fact that the slope of these two regression lines were similar may indicate that the σ value in the exercise experiments (*i.e.* in this case a weighed mean of several reflection coefficients) and in the infusion experiments was roughly equal. Perl (1971) concluded that the σ value for glucose in skeletal muscle was about 0.25 and this value might thus, from what has been said, be valid also for the exercise situation. Fig. 29 may therefore be taken to suggest that in the present exercise experiments the "hyperosmolar extraction" varied from about 97 per cent at low, down to about 75 per cent at high capillary flow velocities, implying that the interstitial hyperosmolality during work would have exceeded the plasma hy-

perosmolality of the venous effluent by some 3 to 30 per cent. Although capillary flow velocity varied considerably in the individual experiments, the average flow velocity was found to be comparable for light, moderate and heavy work (*cf.* data for blood flow and CFC in Fig. 21), the mean values for the ratio of flow/ CFC ranging between 1.19×10^3 (light work) and 1.33×10^3 (heavy work). As can be deduced from Fig. 29, the evaluation of interstitial hyperosmolality from osmolar changes in the venous effluent may therefore have implied an underestimation by some 10—15 per cent on an average, regardless of work intensity. Note that this estimate of the transcapillary "hyperosmolar extraction" during exercise is in close agreement with the conclusion reached in Chapter II (section A1) from experiments in which venous hyperosmolality in exercising muscle was determined before and during mechanical flow reduction.

The present experiments have made it quite clear that the tissue hyperosmolality of exercise leads to pronounced osmotic fluid transfer from the blood stream to the active muscle. In this connection it may be mentioned that considerable transcapillary osmotic fluid shifts have been reported in second degree burns, caused by a secondary developed pronounced tissue hyperosmolality in skin tissue (Arturson and Mellander 1964). In a recent study by Landis and Sage (1971) osmotic fluid transfer across single frog mesenteric capillaries was analysed in response to external hyperosmolality produced by irrigation with hypertonic solutions of, among other substances, NaCl, sodium lactate and urea. These authors found that the transcapillary osmotic fluid shifts were small in relation to the hypertonicity produced and suggested that the transcapillary osmotic reflection coefficients for these substances were in the range of 0.01—0.04, thus far lower than those which may be representative for mammalian skeletal muscle capillaries. This discrepancy may simply be explained by the well-known ultrastructural differences between mesenteric and muscle (as well as skin) capillaries (*e.g.* Majno 1965), the mesenteric ones apparently being much more permeable. It can be argued, however, that the osmotic reflection coefficients for mesenteric capillaries might have been somewhat underestimated in the study of Landis and Sage due to a possible underestimation of the transcapillary osmotic gradient; some solute transfer into the capillary blood at the time of osmotic flux determination thus could have occurred.

In the present experiments several hours elapsed before the fluid accumulated in the muscles during prolonged heavy work had disappeared (Fig. 26). It is conceivable, however, that in the intact organism extravascular fluid drainage *via* the lymph vessels contributes to a more rapid restoration. Evidence was presented to suggest that hydrostatic, colloid osmotic as well as non-protein osmotic forces were responsible for transcapillary fluid absorption after work. The changed hydrostatic pressure gradient after prolonged work was mainly caused by raised tissue pressure but to some extent apparently also by a decrease of capillary hydrostatic pressure below the resting control level, as suggested by the small vessel pressure measurements described in Chapter III. An osmotic fluid

absorption caused by substances other than proteins was indicated by the fact that in the post-exercise period arterial osmolality clearly exceeded venous osmolality, indicating an "osmolar uptake" in the muscle. This process is probably related to the restoration of the energy stores and of the electrolyte balance after work and the substances taken up may partly be same ones that were lost by the muscle during exercise.

In brief summary, the present study has shown that the fluid accumulation in exercising muscle is mainly due to fluid osmosis across the capillary membranes caused by work-induced tissue hyperosmolality which primarily must involve the intracellular space and secondarily affects the interstitium. Thus, at all work intensities, more than 75 per cent of the transcapillary fluid transfer, when maximal, was ascribed to fluid osmosis, the remaining part being due to fluid filtration caused by increased capillary hydrostatic pressure. The experimental findings indicated that the rate of osmotic fluid movement was directly related to the product of the established veno-arterial osmolar difference and blood flow, and this relationship was supported by a theoretical analysis of the data. This analysis of the osmotic process suggested that a "weighed mean" of the transcapillary osmotic reflection coefficients for the substances responsible for the interstitial hyperosmolality was roughly constant at different work intensities. Therefore, the rate of osmotic fluid flux seemed directly related to the established mean transcapillary osmolar gradient and to the transcapillary hydrodynamic conductivity in the region, the latter expressed by the capillary filtration coefficient (CFC). The magnitude of the transcapillary osmolar gradient, in turn, showed a relation to the capillary flow velocity (expressed in relative units by the ratio of blood flow/CFC) through an influence on the transcapillary osmolar extraction. The rate of total fluid flux was correlated to the work intensity. From the relationships mentioned and from the fact that the transcapillary fluid filtration is determined by the product of the increase of capillary hydrostatic pressure and CFC, this can be explained by a correlation between work intensity and tissue hyperosmolality, blood flow, CFC and increase of capillary hydrostatic pressure, respectively (cf. Fig. 21). The main factor limiting the fluid transfer during more prolonged work periods was a gradual decline of the transcapillary osmolar gradient, but additional factors also contributed, *i.e.* increased tissue pressure and increased plasma colloid osmotic pressure. Return of fluid to the circulatory system in the post-exercise period seemed caused by hydrostatic, colloid osmotic as well as by non-protein osmotic transcapillary forces, the latter being related to an *arterial* hyperosmolality created during exercise.

CHAPTER V

**INTERACTION OF CONSTRICTOR MECHANISMS AND HYPER-
OSMOLAR DILATOR EFFECTS IN CONSECUTIVE VASCULAR SEC-
TIONS IN SKELETAL MUSCLE.**

In the intact organism, vascular smooth muscle is subordinated to several different local and remote control systems with either a constrictor or a dilator influence (*cf.* Mellander and Johansson 1968, Mellander 1971). During exercise, for instance, the skeletal muscle vascular bed is exposed not only to local metabolic vasodilator influence but concomitantly also to the vasoconstrictor influence of increased sympathetic vasoconstrictor fibre discharge (*cf.* Bevegård and Shepherd 1967) and, in dependent regions, of myogenic reactions to stretch (*cf.* Lundvall *et al.* 1967). This competitive influence results in characteristic patterns of vascular responses. Thus, it has been clearly established that the resistance effect caused by vasoconstrictor fibre activation is significantly counteracted during exercise (*e.g.* Rein 1930, Remensnyder *et al.* 1962, Kjellmer 1965 *a*), in all probability by the local metabolic dilator influence. On the other hand, the active constrictor response of the capacitance vessels to sympathetic nerve activity is well maintained during work (Kjellmer 1965 *a*). As remarked by Kjellmer, this differentiated pattern of vascular response seems of functional significance, implying that the increased overall sympathetic discharge during exercise is not allowed to impede the nutritional blood supply to the working muscle but acts to decrease venous blood pooling, hence helping to maintain venous return to the heart. Experimental evidence further indicates that a pronounced myogenic constrictor response of the precapillary "sphincters" to increased transmural pressure, present in resting dependent skeletal muscle regions, is relatively well maintained also during exercise, whereas myogenic reactions of the larger precapillary resistance vessels are much impaired during work (Lundvall *et al.* 1967). The myogenic response of the precapillary sphincters, by decreasing the functional capillary surface area, apparently helps to protect against pronounced oedema formation in dependent exercising muscle regions.

A demand to be satisfied by any metabolic factor, alledged that plays an important role in exercise hyperemia, is that in test experiments it should be shown to modify the vascular responses to the adrenergic and myogenic control systems in the same way as exercise. The aim of the present study was to investigate whether this demand was fulfilled by hyperosmolality.

METHODS

25 cats (2.5—4.7 kg) of both sexes, anaesthetized as described in Chapter II, were used in this study.

The investigation was performed on the isolated right lower leg muscles which in some of the experiments were enclosed in a water-filled plethysmograph (38°C) to permit continuous recording of tissue volume and determination of the capillary filtration coefficient (CFC). For surgical and technical details see Chapter II. Arterial inflow pressure and regional venous outflow of blood were recorded continuously. Experiments were performed under a constant pressure perfusion as well as under constant flow perfusion. To permit intra-arterial infusions during constant pressure perfusion the arterial inflow was diverted from the ipsilateral femoral to the popliteal artery through a shunt circuit in which a "mixing chamber" was inserted to ensure thorough mixing with blood (Chapter II). When constant flow perfusion was employed a similar shunt circuit was used and blood flow was controlled by a Harvard perfusion pump.

Experiments involving vasoconstrictor fibre activation. The ipsilateral lumbar sympathetic chain was dissected free and cut centrally. A bipolar platinum electrode was placed at the level of L 4—L 5. The vasoconstrictor fibres were stimulated at 5 V, 5 msec, and at frequencies of 4—16 imp/sec. Atropine (1 mg/kg b.w.) was given to block the sympathetic cholinergic vasodilator fibres.

Isometric muscle exercise was produced by electrical stimulation of the intact sciatic nerve (5 V, 0.5 msec, 1.5—4 imp/sec). These stimuli do not excite the sympathetic fibres to the muscle vascular bed (Chapter II). In most of the exercise experiments the isometrically developed muscle tension was measured by a Grass FT 10 transducer attached to the Achille's tendon. The leg was firmly fixed by pins inserted in the femur and tibia.

Intra-arterial infusions of isotonic and hypertonic (approximately 1500 mOsm/kg H₂O) glucose solutions were made at rates between 0.2—1.0 ml/min using a constant infusion apparatus.

Blood samples (<1.5 ml) were taken for plasma osmolality determinations from a T-tube in the venous outflow tubing close to the popliteal vein after discarding the "dead space" fluid volume. The osmolality was measured and the sampled blood was compensated for as described in Chapter II.

By means of the blood flow and blood pressure recordings the vascular resistance function was followed. The capacitance function was followed by recording the changes of regional blood volume. This was accomplished by external monitoring changes in radio-activity of the red cells labelled with ⁵¹Cr and by simultaneous plethysmographic recording of tissue volume (for details see Åblad and Mellander 1963, Kjellmer 1965 *a*). The radio-activity (100—450 KeV) was recorded by an external scintillation detector (sodium iodide crystal, 1 3/4 inches × 2 inches) fitted in a wide angle lead collimator and registered with a spectrometer connected to a scaler and a linear rate meter (Tri-carb scintillation spectrometer, model 3022, Packard) operating a Rikadenki recorder (model

B-341). The standard deviation setting on the rate meter was 2 %. The radio-activity curve was calibrated with regard to regional changes of blood volume (ml/100 g tissue) by means of concomitant plethysmographic recordings of blood volume increases caused by standardized abrupt adjustments of venous outflow pressure.

Experiments in which myogenic reactions were studied. These studies were performed on the acutely denervated vascular bed of the lower leg muscles. Hypertonic infusions and measurement of plasma osmolality were made as described above. Changes of vascular resistance and of precapillary "sphincter" tone, the latter reflected by alterations of the capillary filtration coefficient (Chapter II), were followed in response to standardized changes of vascular transmural pressure. The experiments were performed under constant pressure perfusion and CFC was determined as described in Chapter II assuming that 80 % of the applied increase of venous outflow pressure was transmitted to the capillary level. Under constant pressure perfusion the CFC determination usually leads to some blood flow reduction as a consequence of the decreased perfusion pressure. Such a blood flow reduction, although small, can induce an error in the CFC determination during hypertonic infusion, since blood flow changes alter the dilution of the infused hypertonic solution in arterial blood and, as a consequence, the magnitude of the transcapillary osmotic fluid absorption caused by the infusion (*cf.* Chapter IV). Therefore, such blood flow changes during the CFC determination were prevented by appropriate adjustments of arterial inflow pressure using a screw-clamp placer around the abdominal aorta. Vascular transmural pressure was varied by 25 mm Hg by simultaneous and equal changes of arterial inflow pressure and venous outflow pressure thus keeping perfusion pressure constant (*cf.* Lundvall *et al.* 1967).

RESULTS

Hyperosmolar versus vasoconstrictor fibre influence. In a first series of experiments (7 animals), performed under constant pressure perfusion, the interaction between experimental hyperosmolality and constrictor fibre activity in the resistance as well as in the capacitance vessels was studied. The constrictor effects of sympathetic stimulation (4—8 imp/sec for 3—4 min) were compared under control conditions and during hypertonic infusion (paired observations at a given rate of stimulation). The control stimulations always caused a marked and well maintained decrease of vascular conductance which averaged 74 ± 4.7 SEM per cent of the vascular conductance prior to the stimulation. Similarly, a well maintained constriction of the capacitance vessels (decrease of regional blood volume) was observed in the control period. This response was partly due to an active constriction of the capacitance vessels and partly to passive elastic recoil as a consequence of a decreased transmural pressure in these vessels resulting from the resistance vessel constriction (*cf.* Öberg 1967). The magnitude of this passive effect was determined in the following way. When vascular conductance

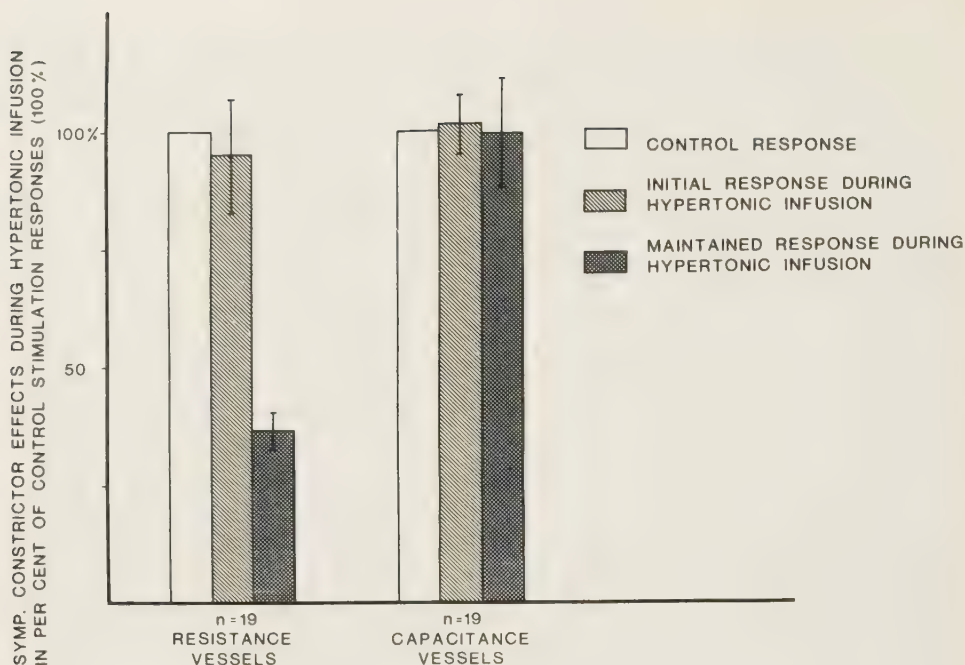


Fig. 30. Diagram showing pooled data of the resistance vessel response (change of vascular conductance) and of the capacitance vessel response (change of regional blood volume) evoked by sympathetic vasoconstrictor fibre activation superimposed on i.a. hypertonic infusion (constant pressure perfusion experiments). The effects are expressed in per cent of observed control stimulation responses.

and regional blood volume had returned after the stimulation period to the control levels, blood flow was mechanically reduced to the same low level as observed during the sympathetic nerve activation. The resulting passive decrease of blood volume is an approximate measure of the passive capacitance response during nerve stimulation (Mellander 1960, Öberg 1967). The blood expulsion caused by *active* constriction of the capacitance vessels could then be calculated as the total minus the passive capacitance response and it averaged 0.29 ± 0.05 SEM ml/100 g tissue, a figure in agreement with the findings of Öberg (1967).

The hypertonic infusions evoked the typical pattern of blood flow response described in Chapter II, A 2, *i.e.* an initial peak flow increase followed by a somewhat less pronounced steady state hyperemic phase. During the steady state response to hypertonic infusion vascular conductance averaged 250 per cent of control vascular conductance and venous osmolality was raised by 25–40 mOsm/kg H_2O . The sympathetic stimulation was superimposed on this steady state response to hypertonic infusion and evoked a characteristic flow response in terms of a marked initial flow decrease after which, during continued stimulation, flow increased again to attain a steady but much less pronounced level of

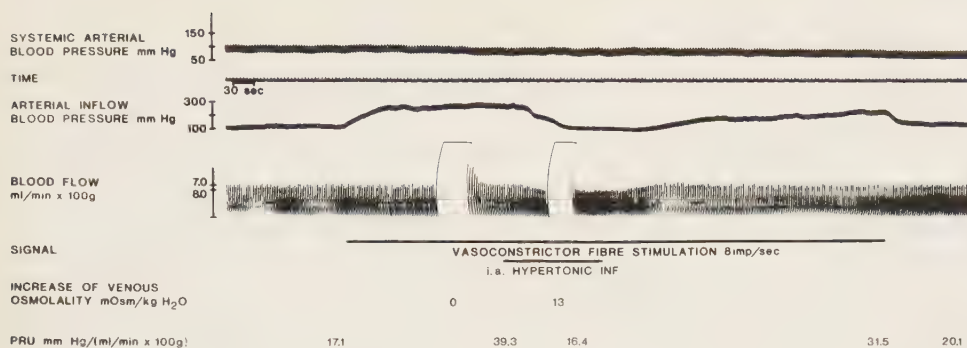


Fig. 31. Record illustrating the inhibitory effect of "experimental hyperosmolality" (i.e., hypertonic glucose infusion) on sympathetic resistance vessel constriction during constant flow perfusion.

flow reduction. This pattern of flow response is very similar to that observed when sympathetic constrictor fibre activation is superimposed on exercise (Kjellmer 1965 *a*, *cf.* also Fig. 33 below). In contrast to the transient resistance response to sympathetic stimulation, the simultaneous constrictor effect in the capacitance vessels was well maintained during the period of stimulation, as shown by a sustained decrease of the regional blood volume (^{51}Cr -technique). After cessation of the nerve stimulation, blood flow and blood volume returned to the pre-stimulatory levels. Then, during continued hypertonic infusion, blood flow was mechanically reduced to the levels observed during constrictor fibre stimulation to permit calculation of the active capacitance response to nerve stimulation (see above).

The results from the described experiments are summarized in Fig. 30 in which the sympathetic constrictor effects in the resistance vessels and the capacitance vessels (active capacitance response) during hypertonic infusion are expressed in per cent of the corresponding control stimulation effects (100 %). The diagram shows mean data $\pm$ SEM for 19 stimulation experiments. It can be seen that the initial (transient) neurogenic constrictor response of the resistance vessels during hypertonic infusion was almost as large as the control response, whereas the maintained constrictor effect averaged only 37 per cent of the control response. The active neurogenic constrictor response of the capacitance vessels, on the other hand, was not decreased during hypertonic infusion. These data thus show that experimental hyperosmolality, like exercise, counteracts quite effectively the response to adrenergic constrictor fibre activation in the resistance vessels but not in the capacitance vessels.

The extent to which graded experimental hyperosmolality interfered with the neurogenic constrictor response of the resistance vessels was investigated in some greater detail in another series of experiments (6 animals) performed under constant flow perfusion. In these experiments, short hypertonic infusions were

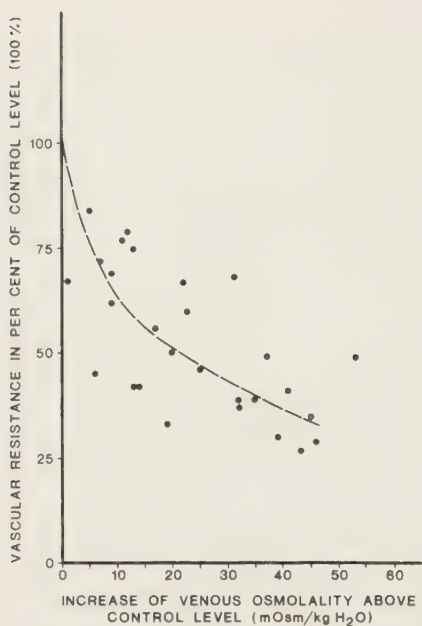


Fig. 32. Diagram showing collected data from the type of experiments illustrated in Fig. 31. Vascular resistance in the early phase of the "steady state" dilator response to hypertonic infusion is expressed in per cent of the resistance prevailing during sympathetic vasoconstrictor fibre activation prior to the infusion and plotted versus the concomitantly observed venous hyperosmolality. Smooth curve drawn by eye.

administered during continuous constrictor fibre activation (8–10 imp/sec). Fig. 31 shows typical tracings from such an experiment. It can be seen that sympathetic stimulation *per se* (8 imp/sec) raised vascular resistance from about 17 to 39 mm Hg/(ml/min $\times$ 100 g). The superimposed hypertonic infusion, which increased venous osmolality by 13 mOsm, abolished this constrictor response, but it reappeared again after the cessation of the infusion.

Fig. 32 shows the collected data ($n=28$) from this series of experiments. Vascular resistance in the early phase of the steady dilator response to hypertonic infusion (*cf.* Fig. 31) is here expressed in per cent of "control" resistance prevailing during sympathetic stimulation prior to the infusion and is plotted against the concomitantly determined venous hyperosmolality. It can be seen that experimental hyperosmolality markedly reduced vascular resistance and in relation to the degree of the induced venous hyperosmolality ($r=-0.67$; $p<0.001$). Sympathetic constrictor fibre activation *per se* increased vascular resistance, on the average, from 18.8 ± 1.56 SEM to 36.3 ± 3.44 SEM mm Hg/(ml/min $\times$ 100 g), *i.e.* by some 100 per cent. It thus follows that at venous hyperosmolality levels exceeding 30 mOsm/kg H₂O (see diagram Fig. 32), the sympathetic constrictor effect was completely abolished (vascular resistance reduced to less than 50 per cent of the "control" response).

In a third series of experiments (7 animals) the resistance function and the regional osmolar changes were followed during intense (8–16 imp/sec) sympathetic stimulation superimposed on exercise. These experiments were performed under constant pressure perfusion and the bulk of the data was obtained during

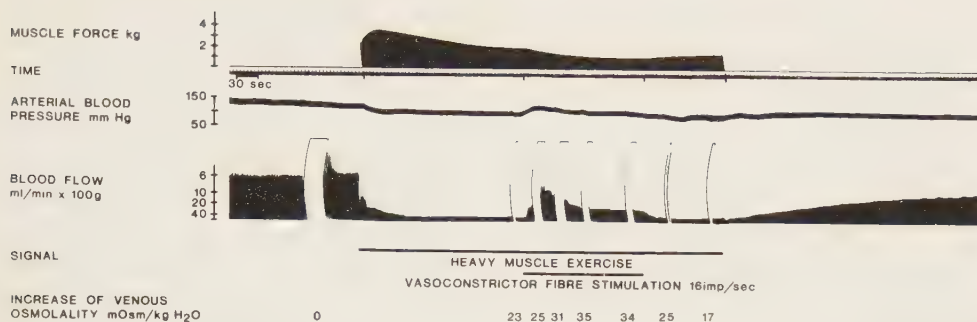


Fig. 33. Typical changes of blood flow, venous hyperosmolality and muscle force induced by intense sympathetic vasoconstrictor fibre activation superimposed on "heavy" exercise.

heavy work (motor fibre stimulation 4 imp/sec) but some observations were also made during moderate exercise (1.5–2 imp/sec). Fig. 33 illustrates tracings from a representative experiment. Exercise at 4 imp/sec increased blood flow to about 40 ml/min $\times$ 100 g tissue. Muscle force was most pronounced initially but tended to decline gradually with time (*cf.* Chapter II, Table I). Venous osmolality had risen by 23 mOsm after 3.5 min of exercise, *i.e.* at the time period when the osmolality induced by work should have reached a maximal level (Chapter II, A1). The superimposed sympathetic stimulation caused an initial marked flow reduction after which flow gradually increased again to a steady level of about 20 ml/min $\times$ 100 g, a pattern of response in agreement with previous observations by Kjellmer (1965 *a*). Note that during sympathetic activation, venous hyperosmolality showed a further gradual rise (up to 35 mOsm) and that this change paralleled the gradual resistance decline. During the sympathetic stimulation the decrease of the muscle force became more marked. Upon cessation of the stimulation, blood flow and muscle force increased again, whereas venous osmolality declined.

The collected data on vascular and osmolar changes (mean values $\pm$ SEM) observed in these heavy exercise experiments ($n=16$) are shown in the diagram of Fig. 34. It can be seen that after 3–4 min of exercise, vascular conductance had increased to some 600 per cent of the resting control value and that venous osmolality had risen by an average of 15 mOsm. Superimposed sympathetic constrictor fibre activation caused an initial pronounced decrease of vascular conductance so that the exercise hyperemia was fully counteracted, but after this, vascular conductance increased gradually and averaged 220 per cent of control conductance towards the end of the stimulation period. Note the gradual further increase of venous hyperosmolality (up to a level of 22 mOsm, *i.e.* an increase of some 50 %) paralleling the latter conductance change. Upon cessation of the vasoconstrictor fibre stimulation, there was a gradual increase of blood flow and a decrease of osmolality. During moderate exercise the pattern of response was

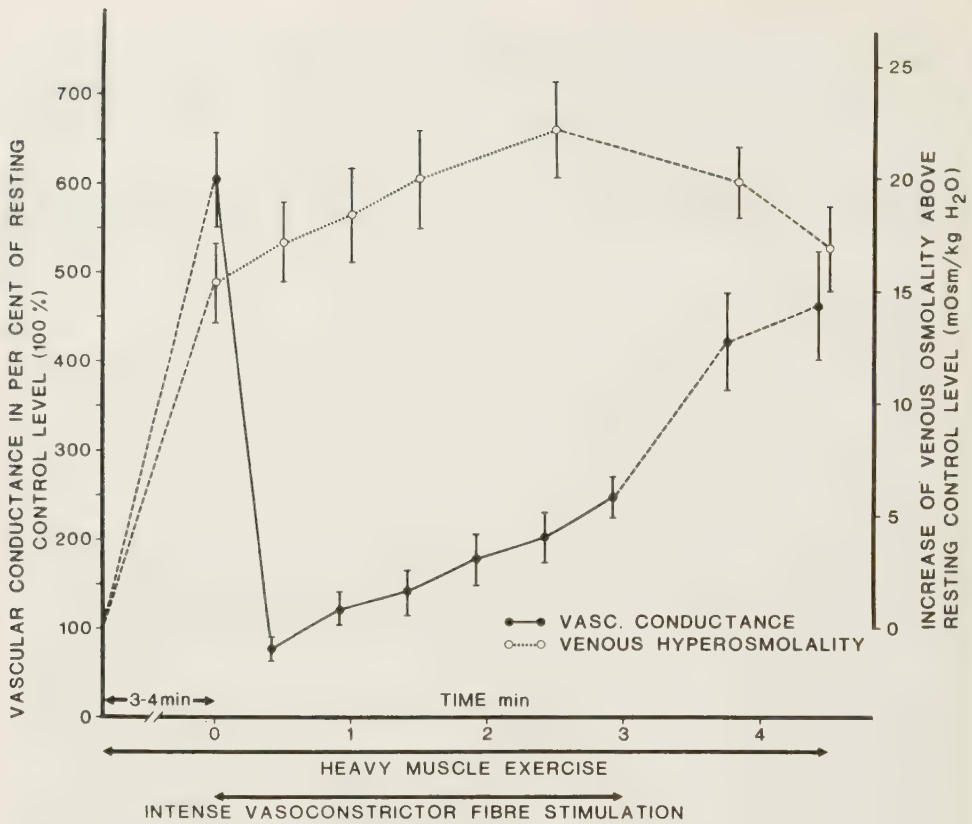


Fig. 34. Diagram showing collected data on changes of vascular conductance and venous hyperosmolality during heavy exercise and superimposed intense sympathetic nerve stimulation. Each point represents mean $\pm$ SEM of 16 observations.

similar but the relative increase of venous osmolality during sympathetic stimulation was more pronounced (about 100 per cent increase).

The data taken together from the described three series of experiments indicate that tissue hyperosmolality interferes with the sympathetic constrictor influence on the resistance vessels during exercise and that a gradual enhancement of tissue hyperosmolality of exercise *per se* during sympathetic activation may reinforce this inhibitory action. The flow reduction caused by sympathetic stimulation seems mainly responsible for the gradual increase of osmolality during exercise, since mechanical flow reduction during work was found also to lead to a similar osmolar increase. Apparently, the neurogenic flow reduction interferes with tissue nutrition, as indicated by the concomitant decrease of muscle force, and may in turn cause an increased anaerobic metabolism and, hence, an increased osmolality, for instance by greater lactate production. The increased osmolality may also be due to a reduced osmolar wash-out from the tissue as blood flow decreases. To

some, but, on average, probably only to a relatively small extent the observed increase of venous osmolality may reflect an improved transcapillary osmolar extraction (*cf.* Chapter IV) and thus not a true increase of interstitial osmolality.

Hyperosmolar versus myogenic reactions. The reactions of the resistance vessels and of the precapillary "sphincters" were studied in the muscle vascular bed in response to increased transmural pressure (5 animals). The arterio-venous pressure gradient was kept constant and transmural pressure was varied by 25 mm Hg by equal shifts of both arterial inflow and venous outflow pressures. High transmural pressure did not cause any augmentation of blood flow in the resting control state (autoregulation in the resistance vessels), but decreased the capillary filtration coefficient (CFC), on the average by 33 per cent, indicating a myogenic constriction of the precapillary sphincters (10 observations). During hypertonic infusion, which raised venous osmolality by some 30 mOsm, high transmural pressure increased blood flow above the level at low transmural pressure indicating impaired autoregulation in the resistance vessels, whereas CFC still decreased by an average of 38 per cent (16 observations). Papaverine infusion superimposed on the experimental hyperosmolality eliminated the latter effect indicating that the described decrease of CFC was due to an active response of the precapillary "sphincters". It thus appears that the dilator action of experimental hyperosmolality can override the myogenic response in the resistance vessels (when examined as the overall resistance function), whereas the myogenic constrictor response of the smallest precapillary vessels (precapillary "sphincters") is still maintained.

COMMENTS

The results presented indicate that during experimental hyperosmolality the vascular response patterns to the adrenergic and myogenic control systems are modified in much the same way as is known to occur during exercise (Kjellmer 1965 *a*, Lundvall *et al.* 1967). These findings lend support to the view that tissue hyperosmolality is a mediator of exercise hyperemia. The efficiency of experimental hyperosmolality, of magnitudes similar to those encountered in exercise, in counteracting the resistance effect to adrenergic vasoconstrictor fibre activation (Figs. 31 and 32) may suggest that the corresponding counteracting effect of exercise itself, in fact, can to a significant extent be related to the work-induced tissue hyperosmolality. The finding that sympathetic vasoconstrictor fibre activation superimposed on exercise caused a clear-cut increase of tissue osmolality in excess of that produced by work alone (Figs. 33 and 34) and that this osmolar change paralleled the gradual decline of the constrictor effect, strengthens this suggestion. It should be pointed out, however, that these observations were obtained during such an intense vasoconstrictor fibre activation that it led to a relatively well maintained blood flow impediment. Experiments using more physiological stimulation rates and different work intensities might reveal whether the phenomenon of adrenergic reinforcement of hyperosmolality can be of significance during normal exercise in the intact organism.

It should be stressed that other metabolic factors than hyperosmolality in all probability can counteract the sympathetic constrictor influence of the resistance vessels during exercise. Thus it has been shown that sympathetic vasoconstriction in resting muscle is much lessened or even abolished during hypoxia and during perfusion with blood with various combinations of hypoxia, increased potassium concentration and hyperosmolality (Skinner and Costin 1969, 1970, 1971). These three factors, which all seem involved in the exercise hyperemia response, were shown to counteract the sympathetic constriction synergistically.

CHAPTER VI

AN EVALUATION OF POSSIBLE TRANSINTIMAL DILATOR EFFECTS
DURING HYPERTONIC INFUSION

A model study on the vascular bed of the intestine

The role of hyperosmolality in exercise hyperemia was evaluated in Chapter II by comparing vascular conductance changes and venous osmolar changes during exercise and during "experimental hyperosmolality", the latter produced by short close i.a. infusions of hypertonic solutions to resting muscle. It was thus assumed that not only during exercise but also during hypertonic infusion vascular smooth muscle was affected by the hyperosmolality created in the interstitium and reflected in the venous effluent. To be able to produce the same rapid interstitial osmolar changes by hypertonic infusion as observed during exercise, the osmolality of the arterial blood had to be raised to relatively high levels. Since the vascular effects were evaluated in relation to venous and not to arterial hyperosmolality, the latter being more pronounced, it follows that the importance of tissue hyperosmolality in exercise hyperemia might have been overestimated if there were a significant direct transintimal dilator effect on the smooth muscle from the intraluminal side of the arterial vessels.

The question whether the dilator effect of hypertonic infusion is exerted from the interstitium after transcapillary osmolar exchange or if there is a significant transintimal influence as well, seems very difficult to approach in a "truly" physiological preparation. Some information of relevance to this problem may, however, be obtained from the present "model experiments", although admittedly they deviate from normal physiological conditions.

The experimental approach implied that dilator effects of the resistance vessels to hypertonic infusion were compared during perfusion of a vascular bed in the normal direction from artery to vein (below denoted "forward perfusion") and during perfusion in the reverse direction from vein to artery ("retrograde perfusion"). Such experiments can be performed on the small intestine, because its vascular bed has no or only rudimentary venous valves (*cf.* Alexander 1963). As will be shown this vascular bed, like that in skeletal muscle, dilates in response to hyperosmolality. The idea behind this approach may be outlined with reference to the schematic drawing in Fig. 35. During forward perfusion, hypertonic infusion into the feeding (arterial) vessel produces an increased interstitial osmolality, the extent of which is reflected in the effluent from the vein; concomitantly, the arterial blood osmolality exceeds significantly the interstitial osmolality. During retrograde perfusion the hypertonic infusion into the feeding

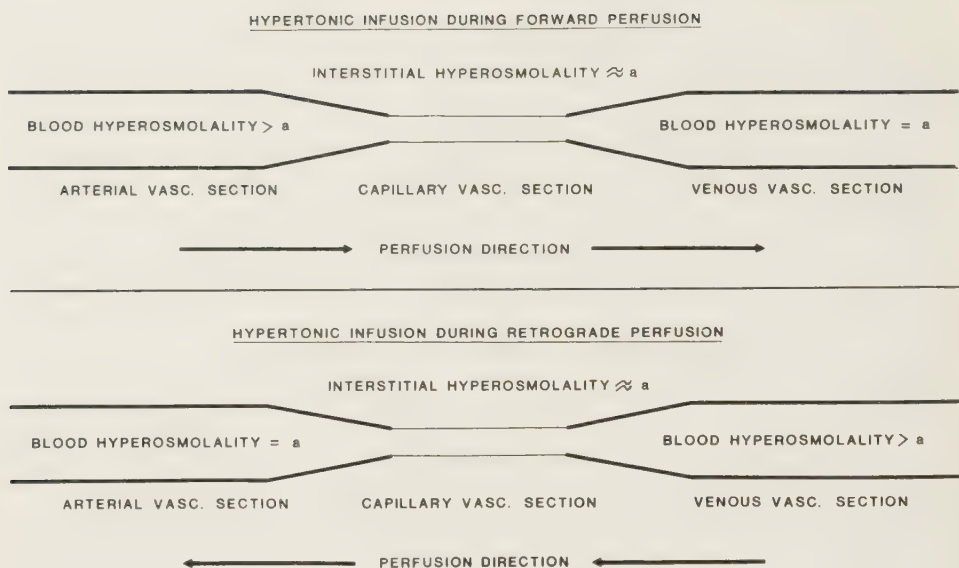


Fig. 35. Relations between osmolality levels in inflow and outflow blood and in interstitium during "forward" and "retrograde" perfusion of the intestine in hypertonic infusion experiments.

(venous) vessel can be adjusted so as to cause the same degree of interstitial hyperosmolality, now reflected in the effluent on the arterial side; concomitantly the osmolality of the blood passing the venous section is higher. Interstitial osmolality can thus be raised to similar levels in both types of perfusion but the intraluminal osmolality in the arterial vessels, the main place of resistance in both experiments (see below), will be considerably greater during forward than retrograde perfusion. It follows that at comparable increases of the osmolality in the outflows, any significant transintimal osmolar resistance effect would be revealed as a greater dilator response during forward than retrograde perfusion; if, on the other hand, similar dilator effects were elicited, hypertonic infusion would seem to exert its vascular effect mainly from the interstitial space after transcapillary osmolar exchange.

METHODS

Experiments were performed on 17 cats of both sexes weighing 2.6 — 4.5 kg and anaesthetized as described in Chapter II. Body temperature was maintained at 37.5—38.5°C using a heating pad and a heating lamp.

The preparation used was basically that described by Folkow *et al.* (1963). The abdomen was opened in the midline and the greater omentum and the spleen extirpated. A section of the jejunum-ileum, weighing 15 — 55 g, was used for the study, the remaining part of the intestinal tract being removed. Care was taken to leave the superior mesenteric artery and vein with their vascular

branches to the intestinal segment intact. The vascular bed of the segment was denervated by cutting the nerve plexuses surrounding the superior mesenteric artery. After heparinization (3 — 5 mg/kg b.w.), a siliconized shunt tubing was inserted which diverted flow from the right femoral artery to the intestine *via* the cannulated superior mesenteric artery. The superior mesenteric vein was cannulated and the venous outflow from the region was measured continuously by diverting flow through an optical droprecorder unit after which flow was returned to the animal *via* a funnel connected to the right jugular vein. Venous outflow pressure was determined by the height above the preparation of the orifice of the outflow tubing. The hypertonic infusions were given proximal to a specially designed "mixing chamber" inserted in the inflow tubing to ensure thorough mixing with blood (see Chapter II). Inflow blood pressure was monitored from a T-tube close to the feeding vessel. Retrograde perfusion was accomplished simply by shifting the arterial and venous tubings at the cannulation sites of the mesenteric artery and vein. As will be described, retrograde perfusion was only possible if inflow pressure was reduced and this reduction was achieved by a screw-clamp placed around the abdominal aorta.

In some experiments the intestinal segment was enclosed in a perspex plethysmograph filled with Tyrode's solution (38°C) to allow continuous recording of changes in tissue volume and determinations of the capillary filtration coefficient (CFC); see Folkow *et al.* 1963.

Isotonic and hypertonic (≈ 1700 mOsm/kg H_2O) glucose (or sometimes sucrose or NaCl) solutions were infused at rates between 0.2 — 3.4 ml/min using a constant infusion apparatus. Plasma osmolality was determined by cryoscopy (Chapter II) in blood samples withdrawn from T-tubes in the shunt circuit distal to the mixing chamber (arterial blood) and in the outflow tubing (effluent blood). After the osmolality determination, the sampled blood was returned to the animal to minimize the blood loss.

Isoprenaline, used as a reference substance to induce a maximal resistance vessel dilatation, was infused in a dose of 0.4 — 0.8 $\mu\text{g}/\text{min}$ (*cf.* Folkow *et al.* 1963); Mechanical interference with blood flow from intestinal smooth muscle activity was avoided by administration of atropine (1 mg/kg b.w.) in the beginning of the experiment. This also minimized secretory activity. Tissue weight was determined for a segment of small intestine of equal length to that under study.

RESULTS

Three different types of experiment were performed and the results will be presented in separate sections. In the first section, (*A*), the reactions of the intestinal resistance vessels to graded hypertonic infusion under "normal" experimental conditions will be described. The second section, (*B*), describes conditions under which retrograde perfusion can be performed and, further, the resistance distri-

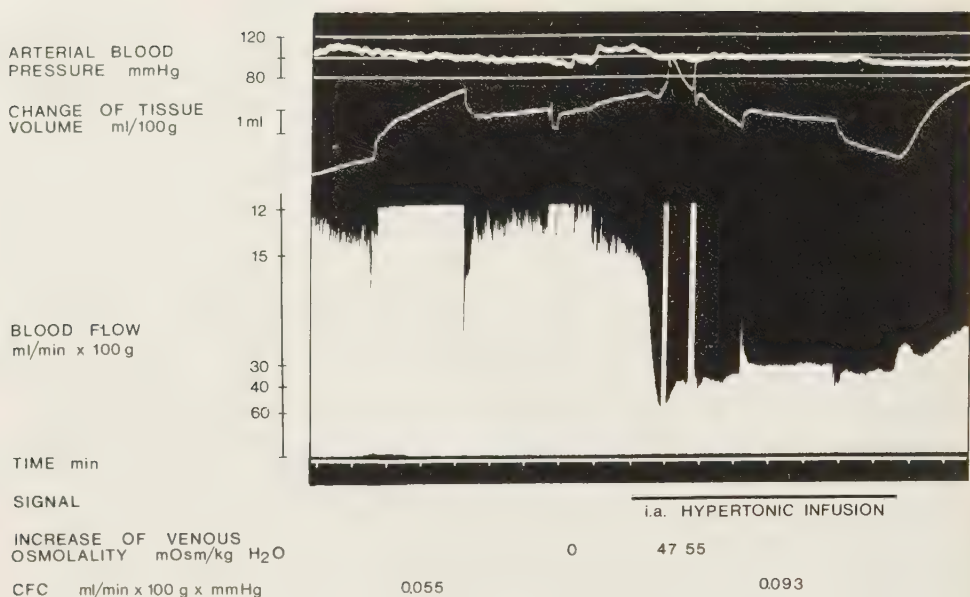


Fig. 36. Changes of blood flow and CFC produced in the small intestine by intra-arterial hypertonic infusion. Flow increased rapidly to a peak value and then decreased during continued infusion to attain an approximately steady state level of hyperemia. Changes of venous osmolality and of CFC indicated at bottom of the figure.

bution between the arterial and the venous side of the vascular bed during forward and retrograde perfusion. Finally, in the third section, (C), the problem whether hyperosmolality during hypertonic infusion influences vascular smooth muscle from the interstitial space or directly from the blood stream was approached.

A. Fig. 36 shows the typical pattern of blood flow changes to hypertonic infusion observed during perfusion in the normal direction and at a normal pressure head. It can be seen that blood flow, which was about 14 ml/min x 100 g tissue in the control period, increased rapidly after the onset of the hypertonic infusion (glucose solution) to reach a maximal value of about 60 ml/min x 100 g within 30 sec ("peak response") after that it decreased somewhat to attain an approximately steady hyperemic level of about 40 ml/min x 100 g ("steady state response") during continued infusion. At the peak response venous osmolality, reflecting interstitial osmolality, was raised by 47 mOsm/kg H₂O, and in the early steady state period by 55 mOsm above the control level. Upon cessation of the infusion blood flow gradually declined towards the control level. The pattern of response of the resistance vessels to hypertonic infusion is thus similar to that observed in skeletal muscle (*e.g.* Figs. 5 and 6, Chapter II). The increased CFC during hypertonic infusion (also noted in the other plethysmo-

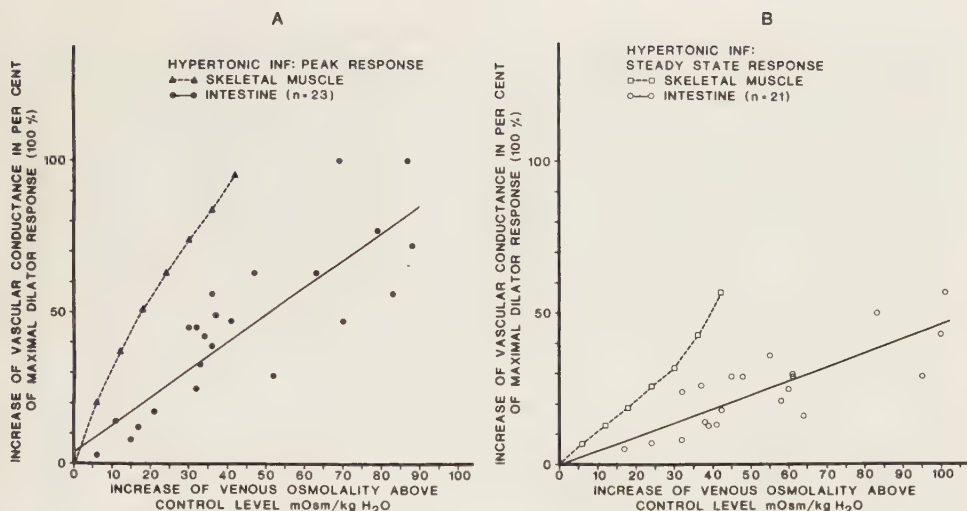


Fig. 37. Diagram showing "peak" and "steady state" dilator responses of the intestinal resistance vessels during i. a. hypertonic infusion against the concomitant increase of venous osmolality (linear regression lines drawn). Osmolar conductance effects expressed in per cent of the maximal conductance increase possible to evoke by i. a. infusion of a supramaximal dose of isoprenaline. Data refer to experiments in which the intestine was perfused at a normal pressure head. For comparison corresponding osmolar effects evoked in skeletal muscle are shown.

graphic experiments) suggests a concomitant dilatation of the precapillary sphincters as in skeletal muscle (*cf.* Chapter II); (It should be mentioned that the CFC determination during infusion could be made under constant pressure perfusion in the intestine which proved difficult in skeletal muscle (Chapter II). This discrepancy seemed related to the fact that osmotic transcapillary fluid absorption was found to be less pronounced in the former than in the latter tissue).

The dilator effects of the intestinal resistance vessels to graded experimental venous hypersmolality, determined during the peak response and in the early phase of the steady state response (*cf.* Fig. 36), were investigated in 6 animals. In each preparation, repetitive infusions were given at intervals permitting full recovery of vascular tone in between the infusions. In the individual animal there was a relation between the magnitude of the dilator response and the degree of hyperosmolality both with regard to the peak and the steady state response.

Considerable variation of the vascular tone in the control state in preparations from different animals (vascular conductance 0.13 — 0.67, mean value 0.31 (ml/min $\times$ 100 g)/mm Hg) made it difficult to make a quantitative evaluation on the whole material since, as discussed in Chapter II, the magnitude of a dilator response is influenced by the control tone. The material in the present

study was too small to compensate adequately for this variation in the way described in Chapter II. In an attempt to circumvent this difficulty, the hyperosmolar dilator effects (vascular conductance during hypertonic infusion minus control vascular conductance prior to the infusion) were expressed in per cent of the maximal dilator response (vascular conductance at maximal vasodilatation (0.96 ± 0.133 SEM (ml/min $\times$ 100 g)/mm Hg) minus control vascular conductance prior to the hypertonic infusion) evoked repetitively in each animal by a short intra-arterial infusion of a supramaximal dose of isoprenaline (Fig. 37). It can be seen that the dilatations elicited both at the peak and at the steady state response were correlated to the degree of venous hyperosmolality ($r=0.82$, $p<0.001$ and $r=0.81$, $p<0.001$, respectively). Both the peak and the steady state vascular responses seem roughly linearly related to the increase of venous osmolality as depicted by the drawn regression lines. For comparison, corresponding data for skeletal muscle, recalculated from Fig. 9 in Chapter II, are shown (broken lines). As a measure of maximal dilatation in skeletal muscle the data during heavy work were used. It is clear that at comparable levels of venous hyperosmolality, the dilatations in skeletal muscle are significantly more pronounced than those in the intestine, both with regard to the peak and to the steady state response. Nevertheless, since the intestinal dilator effects are clear-cut and correlated to the induced hyperosmolality, a basic prerequisite for use of the intestine to approach the problem of possible transintimal influence of hypertonic infusion on the vascular smooth muscle is fulfilled.

B. Pilot experiments showed that the intestinal vascular bed could be perfused for a considerable length of time in the retrograde direction provided inflow pressure was reduced down to the range of 40 — 50 mm Hg. At a normal pressure head, on the other hand, flow virtually stopped after a few minutes, an observation in accordance with that of Alexander (1963). Fig. 38 shows a typical flow pattern during forward perfusion (Panel A) and during retrograde perfusion (Panel B) at the same pressure head (inflow pressure approximately 45 mm Hg and outflow pressure 2 mm Hg). It can be seen that in the initial period during retrograde perfusion blood flow was only moderately reduced compared to the level at forward perfusion. There was, however, some gradual decline with time, probably due to the progressive oedema formation evidenced by the increase of tissue volume. The last part of Panel B shows that blood flow almost stopped when perfusion pressure was raised to about 110 mm Hg; (at the arrow the rising rate for the blood flow ordinate writer was reduced; blood flow was thus in fact the same as just before the arrow). This effect could tentatively be ascribed to a myogenic constrictor response; a critical rise of tissue pressure seems to be a less likely explanation since, after an initial capacitance response, the fluid filtration rate was about similar to that during the low pressure head (see volume tracing).

A prerequisite for the study in section C below is that the resistance distribution between arterial and venous vessels is similar during forward and retro-

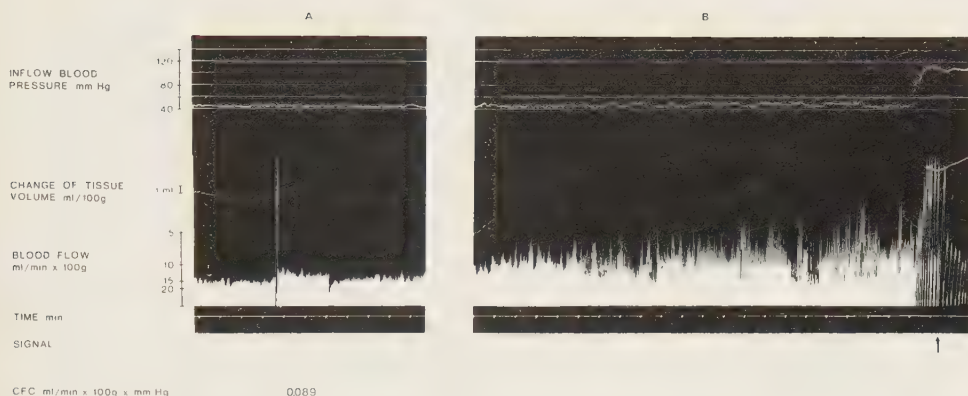


Fig. 38. Intestinal blood flow and tissue volume during “forward” perfusion (panel A) and “retrograde” perfusion (panel B). Retrograde perfusion only possible during reduced inflow pressure. For further explanation see text.

grade perfusion. A deduction of the pre-/postcapillary resistance ratio can be performed with the aid of the volume curve and the CFC determination (regardless of perfusion direction, precapillary resistance will below refer to the arterial and postcapillary to the venous resistance). In the isovolumetric state during forward perfusion at a normal pressure head (about 100 mm Hg), mean hydrostatic capillary pressure (P_c) in the intestine has been estimated to be about 15 mm Hg (Folkow *et al.* 1963), a value adopted for the present deductions. Under these circumstances the pre-/postcapillary resistance ratio is about 6:1. When inflow pressure was reduced an approximate isovolumetric state was still present (see Fig. 38, Panel A) indicating that P_c was maintained at about 15 mm Hg. This P_c value gives a pre-/postcapillary resistance ratio of 2.3:1 (inflow pressure 45 mm Hg and outflow pressure 2 mm Hg), which in turn permits the determination of the CFC which was 0.089 ml/min x 100 g x mm Hg. Since the slight tissue volume decline in Panel A (0.16 ml/min x 100 g) indicated some deviation from a true Starling equilibrium, a more correct value for P_c may be 13 mm Hg (15 mm Hg $-(0.16/0.089)$ mm Hg); this gives a corrected value for pre-/postcapillary resistance of 2.9:1 (note that the CFC-value is hardly affected by such a small correction of the resistance ratio). Reduction of perfusion pressure during forward perfusion, which led to some decrease of overall resistance, was thus associated with a decline of the pre-/postcapillary resistance ratio, apparently due to efficient autoregulatory adjustments mainly in the precapillary resistance vessels. The major resistance is, however, still confined to the last-mentioned vascular section.

The observed filtration rate in the early (<5 min) phase of retrograde perfusion indicated that P_c had risen by about 20 mm Hg compared to the preceding forward perfusion, assuming that CFC was the same as before. Thus P_c would be 33 mm Hg giving a pre-/postcapillary resistance ratio of 2.6:1. The

resistance distribution along the vascular bed during normal and retrograde perfusion at reduced inflow pressure therefore apparently is quite similar, a conclusion reached also in the other experiments of this type. Mention may be made that CFC during retrograde perfusion possibly could have been somewhat lower than assumed due to myogenic constrictor mechanisms. If so, the resistance ratio would have been even higher. The important thing for the analysis in section C is, however, that the pre-/postcapillary resistance ratio during retrograde perfusion is not significantly *lower* than during normal perfusion.

C. In this section a quantitative comparison of the resistance vessel response to hypertonic infusion during forward and during retrograde perfusion was made (8 animals), both types of perfusion being performed at reduced inflow pressure (40 — 55 mm Hg). In the same animal, periods of forward perfusion alternated with relatively short periods of retrograde perfusion. The marked oedema formation occurring during prolonged retrograde perfusion (Fig. 38) was thereby minimized. The hypertonic infusions also helped to limit the oedema formation as a result of the evoked osmotic fluid absorption.

The level of vascular tone present in the intestine during forward and retrograde perfusion could be determined by administering a supramaximal dose of isoprenaline. The control vascular conductance before hypertonic infusion averaged 0.35 ± 0.030 SEM (ml/min $\times$ 100 g)/mm Hg during forward perfusion ($n=25$) and 0.23 ± 0.028 SEM (ml/min $\times$ 100 g)/mm Hg during retrograde perfusion ($n=19$). Vascular conductance during forward perfusion was thus 52 per cent higher than during retrograde perfusion. Vascular conductance during isoprenaline infusion averaged 0.74 ± 0.062 SEM (ml/min $\times$ 100 g)/mm Hg during forward perfusion and 0.48 ± 0.050 SEM (ml/min $\times$ 100 g)/mm Hg during retrograde perfusion, *i.e.* the difference during maximal dilation was in fact almost exactly the same (54 %) as in the control situation. The lower vascular conductance during retrograde perfusion than during normal perfusion both in the control state and at maximal dilatation apparently must be ascribed to some physical factor(s). Note that, despite this difference, the relative vascular conductance increase from the control level in response to isoprenaline was equal (≈ 110 %) during both types of perfusion, indicating that the level of "active" vascular tone in the control state was of comparable magnitude in both cases.

Hypertonic infusion was found to elicit a clear-cut resistance vessel dilatation both during forward and retrograde perfusion, as illustrated in Fig. 39. During normal perfusion (Panel A) a venous osmolality increase by 65 mOsm was associated with an initial blood flow increase from about 11 ml to about 20 ml/min $\times$ 100 g tissue after which flow declined somewhat (steady state response) during the continued hypertonic infusion. Upon cessation of the infusion, blood flow gradually returned to the control level. A later administered supramaximal dose (5 μ g) of isoprenaline raised blood flow to about 23 ml/min $\times$ 100 g tissue at the same pressure head. This response could be considered to represent a ma-

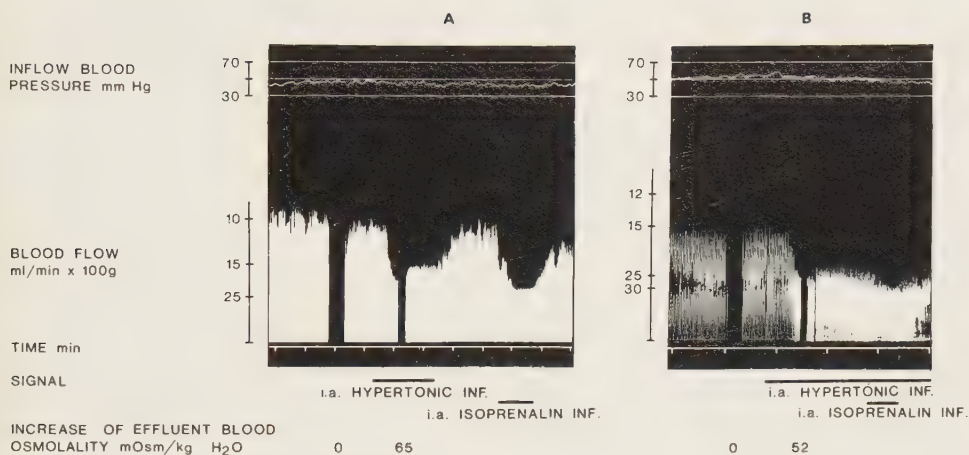


Fig. 39. Resistance vessel dilatations evoked by hypertonic infusion during forward (panel A) and retrograde (panel B) perfusion of the intestine. Observed increases of osmolality in the effluent blood indicated. Maximal vasodilatations evoked by isoprenaline.

ximal resistance vasodilatation (see above). The peak dilator response to hypertonic infusion thus corresponded to about 75 per cent of the maximal dilator response (calculated as described for Fig. 37). During retrograde perfusion (Panel B) an increase of the osmolality in the effluent by 52 mOsm was associated with an initial blood flow increase from about 15 ml to about 25 ml/min x 100 g after which there was some decrease of the hyperemia. This dilator response was 60 per cent of the maximal dilator response evoked by the superimposed isoprenaline infusion taking the slight but significant pressure changes into account. The magnitude of the hyperosmolar dilator responses during the two types of perfusion are not very different in view of the somewhat different osmolalities produced (*cf.* also Fig. 37). Mention may be made that during reduced inflow blood pressure the peak to steady state pattern of flow response was in general clearly less pronounced than in the experiments described in section A above in which the intestine was perfused in the normal direction at a normal pressure head (*cf.* Chapter II, A2).

The collected data from this series of experiments are shown in the diagram of Fig. 40 in which the dilator effects to hypertonic infusion during forward (closed circles, $n = 25$) and during retrograde (open circles, $n = 19$) perfusion are expressed (as above) in per cent of the maximal dilator effect evoked by isoprenaline. The responses are plotted against the induced hyperosmolality of the effluent blood. Correction was made for any rheological effect of the in-

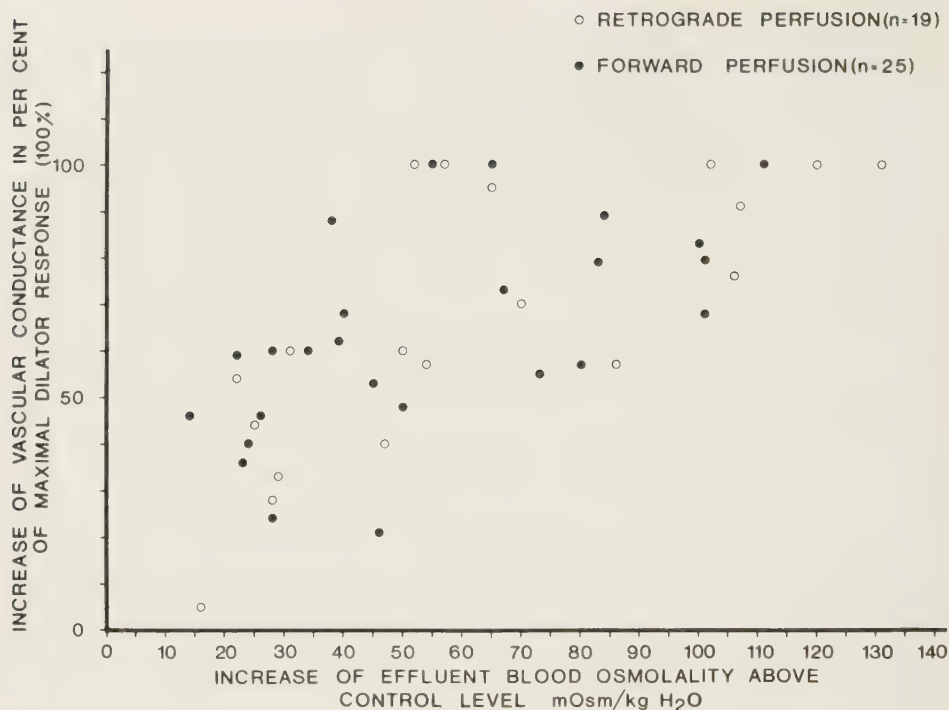


Fig. 40. Diagram showing intestinal resistance vessel dilator responses to hypertonic infusion during "forward" and "retrograde" perfusion against concomitantly observed increases of effluent plasma osmolality. Dilator effects expressed in per cent of maximal dilator response evoked by isoprenaline.

fused fluid *per se*; such effects were revealed by routine isotonic control infusion. The data refer to observations in the early hyperemic phase during the hypertonic infusion because a possible transintimal dilator influence on the vascular smooth muscle should be best revealed at the time when the difference between the hyperosmolality of the perfusate on the inflow side and of the interstitial fluid ($\approx$ outflow hyperosmolality) was most pronounced (*cf.* description to Fig. 35). This osmolar difference of course gradually declined with time both during forward and retrograde perfusion. Both during forward and retrograde perfusion there was a correlation between the dilator response and the evoked hyperosmolality of the effluent blood ($r = 0.60$, $p < 0.01$ and $r = 0.74$, $p < 0.001$, respectively). There was no significant difference between the dilator responses during forward and during retrograde perfusion at any of the hyperosmolality levels produced as shown by linear regression analysis (Scheffe's method, Miller 1966; *cf.* Chapter II, A1).

The osmolality of the perfusate on the inflow side was determined during the development of the dilator response to hypertonic infusion and compared to

that on the outflow side determined during the phase when the dilator response was fully developed during forward ($n = 8$) as well as during retrograde ($n = 8$) perfusion. On the average, the inflow hyperosmolality exceeded the outflow hyperosmolality by a factor of 4.1 during forward perfusion and by a factor of 4.3 during retrograde perfusion. Since the main vascular resistance is located in the arterial section both during forward and during retrograde perfusion (Fig. 38), these pronounced differences between inflow and outflow hyperosmolality should have caused much greater dilator responses during forward perfusion than retrograde perfusion at comparable levels of effluent hyperosmolality if any significant transintimal osmolar dilator effect had been present. However, no such difference between the dilator effects was found (Fig. 40), a fact which indicates that the dilator effect on the resistance vessels to short-term hypertonic infusion is mainly exerted from the interstitial space after transcapillary osmolar exchange.

COMMENTS

Blood-borne vasoactive agents, occurring either naturally in the intact organism or administered experimentally, can gain excess to the vascular smooth muscle cells from the interstitial space of the organ after transcapillary exchange or directly from the blood stream *via* penetration of the intima and associated structures of the vascular wall ("transintimal effect"). In larger, conduit vessels, the existence of vasa vasorum provides another possibility, but in smaller species (adult b.w. <5–6 kg), like the cat, no vasa vasorum seem present, at least not in the small arteries (*cf.* Somlyo and Somlyo 1968). Little seems to be known about which of the routes to the target cells, the transcapillary or the transintimal, is the most important one and, apparently, it may differ with regard to the characteristics of the vasoactive agent in terms of its ability to penetrate membrane barriers, as determined, for instance, by molecular size and degree of lipid-solubility. The problem may become of significance in experimental work. For example, studies of local regulation of blood flow like the search for the factor(s) responsible for functional hyperemia in skeletal muscle, usually involve intra-arterial administration of vasoactive agents. The question then arises whether the evoked vascular effects should be related to the induced interstitial (venous) concentration of the particular substance, as is usually done, or to the arterial blood concentration. Since the latter generally exceeds the venous concentration, at least in short-term infusion experiments, this question is an important one.

The present study was performed in an attempt to investigate this problem with regard to hyperosmolality, produced by intra-arterial hypertonic infusion. It was prompted by the fact that, if a significant transintimal dilator effect were evoked, the data presented in Chapter II would have overestimated the role of tissue hyperosmolality in the exercise hyperemia response as explained in the introductory remarks above. Since the problem seemed very difficult to app-

roach under "truly" physiological conditions, special "model" experiments were performed. Admittedly, the experimental approach involved a major unphysiological procedure, *i.e.* "retrograde perfusion" of the intestinal vascular bed. However, by performing this type of perfusion at a reduced inflow pressure and for only relatively short periods of time, which among other things limited marked oedema formation, the vasculature during retrograde perfusion was found to exhibit, in essential respects, similar characteristics to those shown during perfusion in the forward direction at similar levels of inflow pressure. Thus the control state active vascular tone, the pre-/postcapillary resistance ratio as well as the maximal dilator responses which could be evoked were comparable during the two types of perfusion.

The results presented seem strongly to suggest that the vascular dilator effect of short-term hypertonic infusion is mainly exerted from the interstitial space after transcapillary osmolar exchange and that transintimal influence on the vascular smooth muscle is of little importance. The present study therefore supports the validity of the evaluation of the role of regional tissue hyperosmolality for the functional hyperemia in skeletal muscle as depicted by Fig. 14 in Chapter II. The typical resistance response in muscle to hypertonic infusion in terms of a "peak" flow increase followed by a less pronounced "steady state" hyperemic phase (Chapter II) could *a priori* be ascribed to a significant transintimal dilator effect early during the infusion. This because there will initially be a higher intraluminal hyperosmolality on the arterial side before blood flow increases than after, *i.e.* before the dilution of the infused solution is increased. However, this interpretation thus seems denied by the present data. This seems to remove one important objection against using the peak flow response to hypertonic infusion in the quantitative evaluation of the causal role of tissue hyperosmolality in exercise hyperemia (Fig. 14, Chapter II).

Attempts to explain the present findings must necessarily be speculative. The dilator effect of hyperosmolality seems related to an osmotic shrinkage of the vascular smooth muscle cells as shown by several *in vitro* studies (see General Discussion). Such an effect could be brought about *via* transintimal solute transfer from the blood into the interstitium of the vessel wall and/or *via* transintimal attraction of fluid (water) from the wall. It would appear that at least fluid passage could occur and that therefore some transintimal influence might be present. With regard to the *relative* importance of transintimal effects on the one hand and effects exerted after transcapillary osmolar exchange on the other, the permeability characteristics of the intima and of the capillary membranes should be compared. The total capillary surface area no doubt is much greater than the total intimal surface area of the main resistance vessels (small arteries and arterioles) and the permeability per unit surface area, in addition, is probably significantly greater for the capillaries than for the arteriolar intima. It would seem therefore that the capillaries may provide a much faster route for osmolar exchange with the immediate environment of the vascular smooth muscle cells

and thereby explain the present findings. Some transintimal dilator effect, however, can of course not be excluded, especially not during prolonged hypertonic infusion.

Two recent reports deserve consideration in this connection. Duling and Berne (1970) obtained evidence indicating that oxygen easily diffuses across the vascular wall of precapillary vessels. This is not surprising in view of the fact that oxygen is highly lipid-soluble and therefore penetrates membranes much more easily than the lipid-insoluble substances used in the present study. More difficult to explain in view of the present results is the finding of Uchida *et al.* (1967) that the perfusion of isolated small precapillary vessels (o.d. 250—50 μ) with solutions containing vasoactive agents, such as epinephrine, norepinephrine, serotonin, angiotensin and potassium chloride, evoked clear-cut constrictor effects. It appears that these effects were exerted from the luminal side. It might be argued, however, that the transintimal permeability of these isolated vessels, which often were stored for several days before the experiments, could possibly have been abnormally high thus facilitating transintimal exchange. *In vivo*, humoral control of the peripheral circulation might be exerted mainly after transcapillary substance exchange as suggested by the present study.

CHAPTER VII

GENERAL DISCUSSION

Skeletal muscle tissue exhibits a very rapid and pronounced increase of its metabolic rate when work begins. That the raised metabolism leads to an increased number of osmotically active particles in the tissue was suggested a long time ago (*e.g.* Barcroft and Kato 1915, *cf.* Landis 1934). It has also been known for some time that hypertonic infusions can evoke decreased resistance in the peripheral circulation (*e.g.* Marshall and Shepherd 1959, Overbeck *et al.* 1961, Frohlich 1966). However, a direct physiological role of tissue hyperosmolality in vascular control was suggested only recently when experimental evidence was presented to indicate that tissue hyperosmolality was causally linked to the exercise hyperemia response (Mellander *et al.* 1967).

The present study is an extension of our previous work; by detailed analyses of the vascular effects in muscle during hyperosmolality induced by exercise and by hypertonic infusion in resting tissue a quantitative evaluation of the role of tissue hyperosmolality in exercise hyperemia was performed. Another aim was to elucidate the mechanisms responsible for the considerable transcapillary fluid movement that occurs in muscle during and after work, special attention being paid to possible osmotic processes. The first mentioned problem was investigated by different experimental approaches reported in Chapter II, III, V and VI and the latter problem was dealt with in Chapter IV.

Most of the present experimental findings have already been commented upon in the previous chapters, where the results were subjected to a critical evaluation. This part of the paper will therefore only present a short survey and discussion of the main results and conclusions together with some integrative aspects of the exercise hyperemia response and of the transcapillary fluid dynamics during work.

In Chapter II it was shown that muscle exercise was associated with a considerable regional tissue hyperosmolality, the extent of which was estimated in the venous effluent from active muscle (*e.g.* Fig. 2). The hyperosmolality developed almost immediately at the commencement of work and within a few minutes it reached a maximal level. The dilator response of the resistance vessels showed a similar time-course. The extent of venous hyperosmolality during short-term (<5 min) exercise was correlated to the magnitude of the concomitant dilator response of the resistance vessels, both these phenomena, in turn, being correlated to the "intensity" (somato-motor fibre stimulation rate, 0.25—4 imp/sec) of the work (*e.g.* Fig. 4). During "heavy" (4 imp/sec) exercise venous osmolality could exceed that at rest by as much as 40—45 mOsm/kg H₂O. During

more prolonged work, up to 15 min duration, venous hyperosmolality remained at the peak level reached within the first few minutes when exercise was "light" (<1 imp/sec) or "moderate" (1–2 imp/sec), but tended to decline during prolonged "heavy" work, a decline which then ran parallel to a decline of the developed muscle force (Table I). — The bulk of data were obtained during short-term exercise and the main emphasis will be placed on these results.

Analysis of the constituents of the venous effluent from exercising muscle indicated that the hyperosmolality could be ascribed mainly to increased concentrations of sodium and lactate, but also of some other substances, such as potassium (Table II). Release of substances into the interstitial space from the contracting striated muscle fibres and osmotic fluid (water) movement into these cells can explain the changed composition of the extracellular fluid.

In order to analyse in quantitative terms the effects of hyperosmolality *per se* on the resistance function, an "experimental tissue hyperosmolality" was produced in resting muscle by close arterial hypertonic infusion. To be relevant to the above-mentioned problem of exercise hyperemia it was considered important to design these experiments so that the osmolar changes produced in the tissue (again estimated in the venous effluent) resembled those of exercise not only with regard to *magnitude* but also to *time-course*. Experimental hyperosmolality was always associated with a rapidly developing dilator response of the resistance vessels giving an initial peak blood flow increase ("peak response") followed by a somewhat less pronounced steady hyperemia level ("steady state response") indicating a secondary partial recovery of vascular tone during continued infusion (e.g. Figs. 5 and 6). Observations were also obtained during constant flow perfusion but under these circumstances such partial recovery of vascular tone was not observed (Fig. 8). There was a correlation between the magnitude of the dilator response of the resistance vessels and the concomitant increase of venous hyperosmolality both with regard to the peak and steady state response observed during "constant pressure perfusion" and with regard to the resistance decline during constant flow perfusion (Figs. 9–11). At the peak response a virtually maximal resistance vessel dilatation was present at a rise of venous osmolality by 40–50 mOsm, whereas, to maintain a maximal dilatation as a steady state response, venous osmolality had to be raised by more than 60 mOsm.

A critical analysis of the observed effects on the resistance vessels to hypertonic infusion strongly suggested that, after due correction for the effects of the infused fluid *per se* and of the induced transcapillary osmotic fluid absorption, they could be considered to be caused by direct inhibition of vascular smooth muscle tone on an osmotic basis. As will be discussed below this conclusion is strongly supported by *in vitro* studies on vascular smooth muscle. The inhibitory effect on the vascular smooth muscle of hypertonic infusion seemed exerted from the interstitial space after transcapillary osmolar distribution and not to any significant extent from the luminal side of the arterial vessels *via* a "transintimal" influence. The latter possibility seemed refuted by the study performed in

Chapter VI. This analysis was prompted by the fact that in the infusion experiments the hyperosmolality in the arterial blood exceeded that of the venous blood, especially in early phases of infusion; In Chapter II, the vascular effects of hypertonic infusion were, however, evaluated in relation to the induced venous hyperosmolality.

In this connection the pattern of vascular response in terms of a peak response and a later steady state response to hypertonic infusion deserves consideration. Had a significant transintimal osmotic effect been present it could have explained the early pronounced dilatation, but this possibility seemed excluded by the results of Chapter VI. A more likely explanation for the partial recovery of vascular tone during hypertonic infusion (steady state response) was, however, offered by some results described in Chapter III. It was shown here that a clear-cut rise of pressure seemed to develop in the micro-vessels just prior to the transition of the peak to the steady state response (Fig. 18). The pressure increase became pronounced and was maintained during the steady state response but such an effect was not present during exercise nor during the early phase of the peak response. The initial pressure rise could at least partly be ascribed to transcapillary absorption of fluid to the blood stream during hypertonic infusion and a probable decrease of interstitial pressure consequent to the fluid transfer would make the transmural pressure increase quite pronounced. A transmural pressure rise caused by the osmotic fluid absorption can be considered an "experimental artefact".

Circumstantial evidence (Chapter II and III) suggested that the described transmural pressure rise evoked a secondary myogenic constrictor response in the small precapillary vessels during hypertonic infusion which counteracted the true dilator effect of hypertonicity. It follows that the peak dilator response may be considered more relevant than the steady state response when evaluating the quantitative role of tissue hyperosmolality in exercise hyperemia. This conclusion was supported by the fact that the dilator effects during the "peak response" were quantitatively very similar to the effects obtained during hypertonic infusion under constant flow perfusion; Under constant flow, a myogenic response might well not develop since the perfusion pressure drop during dilatation should tend to balance an increase of transmural pressure.

A quantitative evaluation of the role of tissue hyperosmolality in short-term exercise hyperemia was performed by expressing the resistance vessel dilator response during experimental hyperosmolality in per cent of the exercise hyperemia response at corresponding levels of venous hyperosmolality (Fig. 14, Chapter II). When judged from the peak response during hypertonic infusion, or from the constant flow perfusion experiments, tissue hyperosmolality might explain some 30 to 90 per cent of the exercise hyperemia. When judged from the steady state response, on the other hand, the percentage was 15—60. For all three curves (Fig. 14) the percentage increased with increasing osmolality. Since the work induced tissue hyperosmolality was correlated to the work intensity this suggests

that hyperosmolality is of greatest significance during heavy work. Irrespective of which set of data is used for the evaluation, tissue hyperosmolality can be considered an important mediator of exercise hyperemia. — If, as suggested above, the peak and constant flow data are more relevant to the problem than the steady state effects, the role of hyperosmolality is indeed pronounced. When the peak response data and the constant flow perfusion data are considered together with *average* values for tissue (venous) hyperosmolality induced by exercise of different intensities, more than 40 per cent of the exercise hyperemia during light, and more than 60 per cent during heavy short-term exercise can be ascribed to tissue hyperosmolality. It should finally be mentioned that since a complete transcapillary osmolar equilibrium apparently was not established during exercise and during experimental hyperosmolality (Chapter II section A 1 and A 2, Chapter IV), the data given in Fig. 14 may represent a certain underestimation of the role of tissue hyperosmolality in exercise hyperemia.

Any metabolic factor which plays a causal role in exercise hyperemia should, upon administration to the resting muscle in amounts similar to those believed to be established during exercise, elicit the same specific and differentiated pattern of vascular response in all sections of the vascular bed as the exercise itself (*cf.* Kjellmer 1965 *b* and *c*). That this important demand is fulfilled by tissue hyperosmolality is shown by the various types of experiment reported in Chapter II and III. Thus both exercise and experimental hyperosmolality evoked pronounced dilatation not only in the resistance vessels but also in the precapillary sphincters; on the other hand neither exercise nor experimental hyperosmolality caused any significant active dilatation of the capacitance vessels and apparently no change of capillary permeability (Chapter II). In Chapter III a detailed analysis of the resistance (“resistivity”) changes in very delimited parts of the vascular tree was carried out. The pattern of resistivity change during exercise and during the peak response to experimental hyperosmolality was found to be roughly similar.

During exercise the most prominent effect of superimposed sympathetic constrictor fibre activation is a maintained constriction of the capacitance vessels whereas the constrictor effect of the resistance vessels is quite transient, and the main effect of increased transmural pressure is a myogenic constriction of precapillary “sphincters” (Kjellmer 1965 *a*, Lundvall *et al.* 1967). The fact that the adrenergic as well as the myogenic control systems were shown to elicit these differentiated patterns of response during experimental hyperosmolality supports the view that tissue hyperosmolality is involved in the exercise hyperemia response (Chapter V).

The osmolality hypothesis has also been tested in experiments on man (Lundvall *et al.* 1969). During strenuous forearm exercise a considerable increase of regional venous osmolality was found which could amount to almost 30 mOsm/kg H₂O. Infusion of hypertonic solutions into the brachial artery in amounts that raised regional venous osmolality by up to 20 mOsm evoked a clear-cut decrease

of forearm flow resistance, sometimes down to about 25 per cent of the control value. The latter results were essentially confirmed by Overbeck and Grega (1970). The data considered together strongly suggest that tissue hyperosmolality is a causal factor involved in the exercise hyperemia that develops in our own skeletal muscles during voluntary exercise.

Interstitial deposits of ^{133}Xe in resting human or cat skeletal muscle were found to be cleared at faster rates when the tracer was dissolved in hypertonic medium than when it was dissolved in isotonic medium, indicating that a local microvascular dilator response was evoked when the hypertonicity, as in exercise, primarily involved the extravascular space and was thus not produced *via* the blood stream (Lundvall *et al.* 1969, Gray *et al.*, unpublished observations). The clearance rates were related to the extent of local experimental hypertonicity and could be as large as those observed with isotonic Xenon in exercising muscle.

As discussed the present *in vivo* study strongly indicated that the observed vascular effects of hypertonicity were caused by direct inhibition of vascular smooth muscle tone on an osmotic basis. The mechanisms responsible for the inhibitory action of hyperosmolality have been elucidated in extensive studies of the electrical and mechanical activity in isolated, spontaneously active vascular smooth muscle preparations (Mellander *et al.* 1967, Johansson and Ljung 1967, Johansson and Jonsson 1968, Arvill *et al.* 1969, Johansson 1969). In brief, these studies showed that increased osmolality caused pronounced and sustained relaxation of vascular smooth muscle, mainly by inhibiting myogenic pacemaker activity. This effect, in turn, could be ascribed to changes in transmembrane ionic concentration gradients and in membrane permeabilities to ions produced by osmotic reduction of smooth muscle cell volume. Pronounced hyperosmolality also interfered with intercellular propagation and with excitation-contraction coupling. Therefore, hyperosmolality can exert a negative "chronotropic", "inotropic", and "dromotropic" action on vascular smooth muscle. Exercise leads to pronounced hyperosmolality in the interstitium, *i.e.* in the immediate environment of the vascular smooth muscle, by production and release of osmotically active agents from the contracting skeletal muscle fibres. It is likely that this environmental change leads to shrinkage of the vascular smooth muscle cells and to the consequent series of electrical and mechanical events that were shown to produce relaxation in the *in vitro* experiments.

So far the main emphasis has been placed on the vascular effects observed during short-term exercise and short-term hypertonic infusions. The data taken together strongly suggest that tissue hyperosmolality is of importance for the elicitation and the maintenance of the dilator response during short-term exercise. The following observations suggest that tissue hyperosmolality is of importance for the dilator response also during more prolonged exercise. The hyperosmolality, as mentioned above, was found to be maintained at a high level for a considerable length of time during exercise, at least when it was not too strenuous (Chapter II, A1). Further, hypertonic infusions elicited dilator effects throughout

prolonged infusion periods, but the dilator response was then usually maintained at a relatively steady level despite a gradual rise of osmolality (Fig. 7). A myogenic constrictor mechanism could possibly explain this phenomenon as discussed above. Other alternatives, however, deserve consideration. Since the inhibitory action of hyperosmolality on vascular smooth muscle seems related to cell shrinkage, the maintenance of a dilator effect would depend upon the ability of the osmotically active substance(s) to penetrate the vascular smooth muscle cell membrane. *In vitro* thus, urea hyperosmolality caused no clearly detectable shrinkage of the vascular smooth muscle cells and only a transient inhibition of the spontaneous rhythmic activity, whereas sucrose hyperosmolality caused a sustained and maintained reduction of cell volume and an inhibition of "vascular tone" which also was sustained although most pronounced during the first minutes (Johansson and Jonsson 1968, Arvill *et al.* 1969). These differences were explained by the fact that urea, in contrast to sucrose, showed a rapid entrance into the cells. Among the substances tested in the present *in vivo* hypertonic infusion experiments (sucrose, glucose, mannitol, xylose, NaCl, sodium lactate, and urea) urea was the only that showed signs of fairly rapid penetration in terms of relatively transient dilator response (Chapter II, A2). Since work-induced interstitial hyperosmolality to a great extent seems related to increased concentrations of sodium and lactate (Table II), this may imply that vascular smooth muscle shrinkage during exercise is maintained for relatively long periods of time. Despite a maintained smooth muscle cell volume reduction it is possible, however, that there is some recovery of vascular tone as suggested by the described *in vitro* experiments with sucrose; it can not be excluded that such a phenomenon, if present *in vivo*, to some extent could be responsible for the transition from the peak to the steady state response in the earlier phase of hypertonic infusion. Although no definite quantitative evaluation of the role of tissue hyperosmolality during more prolonged exercise can be given at present it is quite clear, however, that the rapid and pronounced changes of tissue hyperosmolality that occur during *short-term* exercise will cause considerable inhibition of vascular smooth muscle tone and, hence, marked vasodilatation as summarized in Fig. 14.

The hypothesis that tissue hyperosmolality is a mediator of exercise hyperemia has, besides in the studies discussed above, been approached also in some other laboratories. Thus, Scott *et al.* (1970) and Scott and Radawski (1971) confirmed on dog skeletal muscle that exercise was associated with increases of regional tissue osmolality which, under conditions of constant flow perfusion, were of similar magnitudes to those reported in the present study and roughly maintained for the period of work. During constant pressure perfusion, however, they found a decline of the work-induced tissue hyperosmolality with time. They also studied vascular effects in resting muscle in response to prolonged hypertonic infusions administered at successively increasing infusion rates and found only moderate dilator effects. From these observations they concluded that tissue

hyperosmolality could scarcely play an important role in exercise hyperemia. It was emphasized above that in order to reveal the true role of tissue hyperosmolality in exercise hyperemia from intra-arterial hypertonic infusion experiments, the infusion rates should be adjusted so as to create the same rapid increase of tissue hyperosmolality as occurs during exercise. In the experiments of Scott *et al.* and Scott and Radawski the hypertonic infusion technique must, however, have led to only relatively slow and gradual increases of tissue osmolality; therefore, because of the poor resemblance to the osmolar events occurring during exercise itself it is probable that their experiments may permit no valid conclusion about the quantitative role of tissue hyperosmolality in exercise hyperemia. Skinner and Costin (1971) and Gazitù *et al.* (1971) also observed relatively moderate dilator effects in skeletal muscle to hypertonic infusion, but, again, this seems related to the fact that, apparently, a fairly slow increase of tissue osmolality was produced in these studies.

As stated in Chapter I, exercise hyperemia is in all probability caused by mutual, synergistic actions of several local metabolic dilator factors. Hyperosmolality appears to be one of these factors, and apparently an important one. Other possible metabolic mediators of functional hyperemia in skeletal muscle have been discussed at great length in different reviews (*e.g.* Barcroft 1963, Mellander and Johansson 1968, Haddy and Scott 1968, Mellander 1970) and therefore a detailed recapitulation of this subject will not be made. To summarize, it appears that, besides tissue hyperosmolality, the following factors deserve special consideration: the potassium ion (Kjellmer 1965, *b* and *c*), oxygen lack (*e.g.* Fairchild *et al.* 1964), inorganic phosphate (Hilton and Chair 1971). For a detailed discussion of the problem the reader is referred to above-mentioned reviews.

Since the metabolic pathways and, hence, the individual "metabolite" concentrations in skeletal muscle differ with regard to the type of exercise performed there is every reason to believe that the relative importance of the different dilator factors can vary, for instance during different phases of the exercise hyperemia response and under different work loads of the skeletal muscle. It follows that the conditions under which a proposed mediator of exercise hyperemia plays its main role should be investigated and defined to be able to correctly evaluate its importance for the reaction. From the present study it appears that, although tissue hyperosmolality seems to play a significant role for exercise hyperemia at all levels and in all periods of work, its relative importance seems to be greatest during the development of the vasodilatation and in the subsequent early phases of exercise when the rate of change of tissue osmolality is rapid and furthermore, perhaps particularly in heavy work when the degree of tissue hyperosmolality is most pronounced. The type of detailed investigation performed here may prove useful for evaluating the relative role played by other metabolic mediators. Up to now only tissue hyperosmolality and potassium have been studied in such detail. An interesting approach to the problem has been used by Skinner and Costin (1970, 1971) who have studied the combined effects of potassium, oxygen

deficiency, and hyperosmolality on the resistance vessels in resting skeletal muscle. The results showed that these factors acted synergistically to enhance the hyperemia response.

In Chapter IV the net transcapillary fluid movement into exercising muscle was dealt with and the mechanisms responsible for this fluid transport as well as transcapillary processes involved in the fluid return to the circulatory system after exercise analysed. The investigation was performed on the lower leg, cat muscles which constitute a relatively small part of the total muscle mass (*cf.* discussion below). The regional lymph vessels were ligated to be able to study transcapillary events only. The data were discussed at length and in relation to relevant literature and will only be briefly summarized. The fluid flux was found to be related to the work intensity in agreement with Jacobsson and Kjellmer (1964). The rate of fluid movement reached a maximum 2—4 min after commencement of work and was then maintained approximately constant during light and moderate prolonged (15 min) exercise whereas it declined gradually during heavy (4 imp/sec) work. The total amount of fluid that accumulated in the lower leg muscles during 15 min of heavy work averaged no less than about 15 ml/100 g tissue (Fig. 25). The investigation provided concomitant data of fluid transfer into the active muscles, of changes of capillary hydrostatic pressure (derived partly from the study reported in Chapter III), of transcapillary hydrodynamic conductivity as determined for the whole muscle region in terms of the transcapillary filtration coefficient (CFC), and of regional osmolar events. The results seemed to make it clear that fluid accumulation in exercising muscle is mainly due to transcapillary fluid osmosis resulting from the work-induced tissue hyperosmolality. Thus, at all work intensities, more than 75 per cent of the fluid flux, when maximal, was ascribed to fluid osmosis, the remaining part being due to fluid filtration caused by increased capillary hydrostatic pressure (Figs. 20 and 21). This figure (>75 %) seemed roughly representative also for the cumulative fluid flux during prolonged exercise. The experimental findings indicated that the rate of osmotic fluid movement was directly related to the product of the established veno-arterial osmolar difference and blood flow, and this relationship was supported by a theoretical analysis of the data. This analysis suggested that a "weighed mean" of the transcapillary osmotic reflection coefficients for the substances responsible for the interstitial hyperosmolality was approximately constant at different work intensities and that therefore the osmotic fluid flux was directly related to the established transcapillary osmolar gradient and to the overall transcapillary hydrodynamic conductivity in the studied vascular bed. The magnitude of the transcapillary osmolar gradient, in turn, showed a relation to blood flow (capillary flow velocity) through an influence on the transcapillary osmolar extraction. The correlation between work intensity and the rate of total fluid flux could be explained by the mentioned relationships and by a correlation between work intensity, tissue hyperosmolality, blood flow, CFC and increase of capillary hydrostatic pressure, respectively.

A gradual decrease of the transcapillary osmolar gradient was the main factor which limited the fluid movement during prolonged heavy exercise. Rough deductions suggested that a decrease of the transcapillary hydrostatic pressure gradient (increased tissue pressure) and an increase of the colloid osmotic pressure gradient (increased plasma colloid osmotic pressure) also contributed. The decreased transcapillary osmolar gradient was due to a gradually diminishing interstitial hyperosmolality and to an increased arterial osmolality, the latter, at least partly, being due to a "delivery of osmols" from the exercising muscles to the general circulation (*cf.* Chapter II, A 1).

The return to the intravascular space after work of the fluid accumulated in the exercising muscles was a slow process which after prolonged heavy exercise required several hours (Fig. 26). Evidence was presented (Fig. 26) to suggest that hydrostatic, colloid osmotic as well as non-protein osmotic forces were responsible for the transcapillary fluid absorption. The non-protein osmotic fluid absorption was related to a maintenance for a considerable length of time of the increase of arterial osmolality that occurred during exercise.

It should finally be stressed once more that in the experiments described, lymph drainage was prevented. Therefore, in the intact organism, fluid accumulation in exercising muscle is probably somewhat less pronounced and the rate of fluid return to the circulatory system somewhat greater.

Hitherto the discussion has been concerned with *regional* effects of the tissue hyperosmolality in active muscle. A few comments will also be given on some *generalized* effects on cardiovascular hemodynamics that can result from work induced hyperosmolality.

First, the effects of hyperosmolality on the plasma volume will be considered. As mentioned, the fluid accumulation in the active muscles averaged about 15 ml/100 g tissue during 15 min of heavy work with a small muscle group (lower leg muscles of the cat). It can be deduced that if fluid accumulated to the same extent in all active muscle during whole body exercise, then the fluid loss from the circulatory system would exceed the entire plasma volume in a relatively short period of time. It is well-known, however, that in man, plasma volume decreases by at most 15 to 20 per cent, or by some 600 ml, during heavy work with large muscle groups (*e.g.* Åstrand *et al.* 1964, Ekelund and Holmgren 1964, Hartley *et al.* 1970). This may imply either that fluid accumulation is much more limited when large than small muscle groups are active, and no greater than the actual plasma volume decrease, or, if more pronounced, that fluid must be transferred into the circulatory system, for instance from the extravascular space of inactive tissues. A recent study (Lundvall *et al.* 1970, 1972) in which the fluid accumulation in the active muscle mass and the change of plasma volume were estimated during short-term bicycle exercise in man suggested that the latter train of events takes place. Thus it was shown that during 6 min heavy exercise on a bicycle ergometer (1200—1500 kpm/min) the volume of the leg in normal males increased so as to indicate, after correction for increased regional

blood volume, an average transcapillary plasma fluid loss into the leg muscles of 4.5 ml/100 g tissue. This fluid accumulation corresponds only to some 50 per cent of that observed in the present study on the lower leg, cat muscles during 6 min of heavy work (*cf.* Fig. 25). Nevertheless, in the human experiments, the total fluid loss into the entire active muscles mass (≈ 25 kg) was calculated to comprise about 1100 ml. Since the concomitant decrease of plasma volume in man was shown to be about 600 ml, it follows that some 500 ml of fluid must have entered the circulatory system during the work. The study indicated that this compensatory fluid gain was accomplished by absorption of extravascular fluid from inactive tissues and partly caused by fluid osmosis resulting from a work-induced pronounced *arterial* hyperosmolality. Thus, the increase of arterial osmolality averaged no less than 22 mOsm/kg H_2O above the resting control level and seemed mainly caused by "delivery of osmols" from the large active muscle mass. In the study referred to fluid absorption from inactive tissues was also studied in experimental animals during exercise and in resting humans during arterial hyperosmolality produced by intravenous hypertonic infusion. These investigations suggested that at least half of the fluid gain to the circulatory system in heavy exercise could be ascribed to the increased arterial osmolality and the remainder to a reflex decrease of capillary pressure. It thus appears that the generalized hyperosmolality in the arterial blood resulting from exercise with large muscle groups puts a powerful osmolar mechanism for plasma volume control into play. The arterial hyperosmolality helps to keep the plasma volume reduction within tolerable limits partly by causing osmotic fluid absorption from inactive tissues and partly by limiting the fluid loss into the active muscles. The latter effect should result from "osmolar recirculation" which decreases the transcapillary osmolar gradient in the working muscles and may mainly explain why the fluid accumulation was less pronounced in exercising muscle when large muscle groups than when small muscle groups were active. Another factor that can help to explain this difference is the fact that in the animal experiments lymph drainage was prevented. In the experiments in man, on the other hand, return of fluid to the circulatory system *via* the lymph vessels must have reduced to some extent the fluid accumulation.

The work-induced hyperosmolality in the active muscle and in arterial blood might also contribute to the general cardiovascular adaptation to exercise. Thus, Lasser *et al.* (1960) obtained evidence to suggest that hypertonicity in skeletal muscle affects locally situated "osmoreceptors" and that thereby a reflex increase of arterial blood pressure and a tachycardia is induced and, in addition, an increased respiratory rate. It was concluded that afferent impulses travel in the peripheral somatic nerves to the central nervous system and induce an increased discharge rate in sympathetic fibres. The degree of tissue hyperosmolality required to elicit these effects are, however, not known. The considerable increase of arterial osmolality occurring during exercise with large muscle groups seems also to affect the central circulation. Thus Hauge and Bø (1971) and Bø

et al. (1971) reported that an arterial hyperosmolality of some 25 mOsm/kg H_2O evoked a decrease of pulmonary vascular resistance and concomitantly a constriction of the pulmonary capacitance vessels. Further, it has been shown that arterial hyperosmolality of the magnitude just mentioned exerted a positive inotropic action on the heart (Wildenthal *et al.* 1969 *a*), ascribed partly to neurogenic mechanisms and partly to a direct effect on the heart muscle. The latter effect was supported by studies *in vitro* (Wildenthal *et al.* 1969 *b*, Koch-Weser 1963).

Thus hyperosmolality during exercise may affect the circulation in many different ways: The tissue hyperosmolality in the active muscles promotes the exercise vasodilatation and is mainly responsible for transfer of plasma fluid into the muscle. It might further stimulate local "osmoreceptors" so as to induce a reflex arterial pressure increase and tachycardia. In the general circulation, work-induced arterial hyperosmolality can establish an important osmolar control of plasma volume by fluid absorption from inactive tissues and by limiting the transfer of fluid into the active muscle so that plasma volume is maintained within tolerable limits. Further, arterial hyperosmolality may promote the action of the heart *via* an inotropic effect, decrease the resistance in the pulmonary circulation and concomitantly constrict the pulmonary veins so as to mobilize blood to the systemic circulation.

GENERAL SUMMARY

Experimental evidence suggests that vasodilatation in exercising skeletal muscle is caused by the synergistic actions of several local dilator factors related to the increased tissue metabolism. The present study, performed on the cat skeletal muscle, indicates that regional tissue hyperosmolality induced by work is an important mediator of the vasodilatation and of the net transcapillary fluid flux in exercising muscle. This conclusion is based on the following main results:

1. Muscle work induced a rapid rise of tissue (venous) osmolality which paralleled the dilator response of the resistance vessels. The extent of hyperosmolality during short-term (<5 min) exercise was correlated to the magnitude of the concomitant vasodilator response, both these phenomena, in turn, being correlated to the "intensity" of the work. During "heavy" exercise venous osmolality could exceed that at rest by 40—45 mOsm/kg H_2O . The hyperosmolality in the extracellular space seemed mainly caused by increased concentrations of sodium and lactate. During more prolonged work the hyperosmolality was maintained except during strenuous work when it tended to decline.
2. Close arterial hypertonic infusions to resting muscle producing graded increases of tissue (venous) hyperosmolality similar to those observed in exercise evoked rapid and graded dilatations of the resistance vessels. Critical analysis indicated that these effects were caused by direct osmotic inhibition of vascular smooth muscle tone by the hypertonicity established in the interstitial space after transcapillary osmolar distribution. Under constant pressure perfusion the dilator response was most pronounced in the initial phase of infusion ("peak response"), after which there was some partial recovery of vascular tone ("steady state response") possibly caused by a secondary myogenic constrictor effect due to increased transmural pressure. Under constant flow perfusion the osmolar dilator response did not show any such phasic pattern.
3. Comparison of the dilator effects of exercise and of hypertonic infusion permitted an approximate quantitative evaluation of the role of tissue hyperosmolality in exercise hyperemia. The relative importance of this factor seemed to be greatest in early phases of work and especially in heavy exercise when the degree of tissue hyperosmolality was most pronounced. The hypertonic infusion effects observed during the "peak response" and during constant flow perfusion could be considered most relevant to the quantitative evaluation. These data suggested that more than 40 per cent of the exercise hyperemia could be ascribed to tissue hyperosmolality during light, and more than 60 per cent during heavy short-term exercise. Hyperosmolality may play an important but perhaps somewhat less pronounced role for the dilatation during prolonged work.
4. Studies of the resistance function with regard to changes in resistance distribution within consecutive, delimited parts of the entire vascular tree, and analyses of the precapillary sphincter function and of the capacitance

function showed that the vascular functions were all affected in much the same way during hypertonic infusion to resting muscle as during exercise. Furthermore, the highly differentiated patterns of response within various consecutive vascular sections known to be evoked when adrenergic or myogenic stimuli are superimposed on exercise were found to be elicited when these control systems were activated during hypertonic infusion to resting muscle. These findings support the view that tissue hyperosmolality is a causal factor in the exercise vasodilatation.

5. The present results considered together with previous detailed studies of the effects of hyperosmolality on mechanical and electrical activity in isolated spontaneously active vascular smooth muscle suggest that the following train of events occur in exercise: Hyperosmolality develops in the interstitial space of active muscle due to production and release of osmotically active particles from the contracting striated muscle fibres and to osmotic uptake of fluid (water) into these cells; the hyperosmolality in the interstitial space, the immediate environment of the vascular smooth muscle, causes osmotic shrinkage of the smooth muscle cells. The consequent changes of smooth muscle transmembrane ionic concentration gradients and membrane permeabilities to ions lead to inhibition of vascular tone and, hence, to vasodilatation.

6. Concomitant direct or indirect observations in working skeletal muscle of net transcapillary fluid movement, of changes of capillary hydrostatic pressure, of capillary hydrodynamic conductivity and of regional osmolar events indicated that the pronounced fluid accumulation occurring in muscle during exercise is caused mainly (>75 per cent) by osmosis as a result of the work-induced tissue hyperosmolality and that the remainder is due to filtration. The transcapillary fluid absorption into the blood stream *after* exercise seemed caused by hydrostatic, colloid osmotic as well as by non-protein osmotic forces, the latter related to an *arterial* hyperosmolality created during the preceding work period.

7. Some implications of a pronounced arterial hyperosmolality evoked when large muscle groups are active are discussed with regard to effects on general cardio-vascular dynamics.

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